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Salinity Effects on the Development and Larval Tolerance of Five Species of Echinoderms.

Richard Allen Roller

Louisiana State University and Agricultural & Mechanical College

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**Salinity effects on the development and larval tolerance of five
species of echinoderms**

Roller, Richard Allen, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1987

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**Salinity Effects on the Development and Larval
Tolerance of Five Species of Echinoderms**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Zoology and Physiology

by

Richard Allen Roller

B.S. University of Arkansas at Little Rock, 1980

M.S. Louisiana State University, 1983

August, 1987

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ABSTRACT

Salinity effects on the developmental rates, larval tolerances and various metabolic processes of five species of echinoderms were investigated. Development of Lytechinus variegatus (Lamarck), Strongylocentrotus droebachiensis (O.F. Muller, 1776), Strongylocentrotus purpuratus (Stimpson, 1857), Strongylocentrotus pallidus (G.O. Sars, 1871), and Pisaster ochraceus (Brandt, 1835) larvae were observed. Developmental rates and larval survival to metamorphosis of S. droebachiensis and S. pallidus varied directly with salinity and were well within the observed salinity tolerance and distributional limits for the adults.

For each species, embryos and larvae at lower salinities (20, 22.5, and 25‰) tended to develop more slowly than those at higher salinities. P. ochraceus and S. droebachiensis survived all salinity treatments throughout the experimental period. F₁ hybrid larvae exhibited salinity tolerances and developmental rates intermediate between values reported for the more stenohaline larvae of S. pallidus and those for the more euryhaline larvae of S. droebachiensis. S. purpuratus larvae were stenohaline and tolerated salinities as low as 27.5‰.

Gonadal ninhydrin positive substances were significantly lower at 17.5‰ and coelomic cavity lactic acid values were significantly higher at 20‰ for S. droebachiensis and S. pallidus, although, histological examination revealed no observable differences in gonadal structure between adults

acclimated to high and low salinity.

Temperature and salinity effects on the development, metabolic rates, and larval tolerance of Lytechinus variegatus (Lamarck) were also examined. Developmental rates and survival to metamorphosis of larval L. variegatus varied directly with salinity. Respiration rates of L. variegatus plutei varied directly with salinity and temperature; whereas, excretion rates varied directly with temperature and indirectly with salinity. O:N ratios suggest increased reliance on protein catabolism thus indicative of physiological stress at 27.5°/ooS. The back transfer of juvenile L. variegatus to low salinities correlates well with 28 day LC₅₀ data of adults indicating 18°/oo to be the low salinity tolerance limit of adult urchins.

Data obtained from this study indicate that the larval tolerances of echinoderms may limit adult distributions along salinity gradients.

INTRODUCTION

Echinoderms have been long considered to be an entirely marine assemblage; however, recent investigations have revealed that several species are found in brackish water and are capable of tolerating relatively wide fluctuations in salinity (Binyon, 1972; Stickle and Denoux, 1976; Sabourin and Stickle, 1981; Shirley and Stickle, 1982). The salinity extremes inhabited by several species varies from 7.7°/ooS for the ophiuroid Ophiophragmus filograneus (Thomas, 1961) to 60°/ooS for the asteroids Astropecten polyacanthus phragmorus and Asterina burtoni (Price, 1982; as reviewed in Stickle and Diehl, 1987). Previously it was believed that brackish water populations of echinoderms were found only in regions, such as the Baltic and Black Sea, where the dilution of sea water has been very gradual, occurring over geological epochs (Binyon, 1966, 1972). Recently however, several populations of echinoderms have been found in regions subjected to seasonal and/or diurnal fluctuations in salinity (Stickle, and Denoux, 1976; Drouin et al., 1985; Stickle and Diehl, 1987).

Marine invertebrate larvae are known to be more sensitive to fluctuations in environmental conditions than adults and are therefore considered the precarious link in the life cycle (Calabrese and Davis, 1970; Watts et al., 1982; Roller and Stickle, 1987). Thorson (1950) identified several sources of larval "wastage" including food availability and the duration of

time in the plankton. The longer a larvae remains in the plankton, the greater its chances of succumbing to predation, starvation, or detrimental environmental conditions such as pollution or salinity and temperature variations. Several investigators have also found echinoderm larvae to be particularly sensitive to low salinity (Thorson, 1946; Fenchel, 1965; Watts et al., 1982). Gezelius (1963) found that the cleavage rate of fertilized Psammechinus miliaris eggs at a given temperature depended on the salinity to which the parents had been adapted. Greenwood and Bennett (1981) performed osmotic shock experiments on the spermatozoa and ova of Paraechinus angulosus by preincubating each at 45 temperature-salinity combinations before fertilization under optimal conditions for maximal fertilization. They found that temperature had the greatest effect on spermatozoa but salinity proved injurious to the ova. Giese and Farmanfarmanian (1963) found that the gastrulae of Strongylocentrotus purpuratus tolerated and differentiated in the same salinity range (28-38.5‰) as that tolerated by the eggs and adults. Watts et al. (1982) transferred 2-day-old type 1 and type 2 larvae of the asteroid Echinaster species complex to three temperatures and three salinities and found that salinity was the dominant factor affecting development and growth. F₁ hybrids showed intermediate development and growth responses at the apparent optimal conditions and they exhibited stronger maternal characteristics. These studies suggest that salinity tolerances and developmental rates of echinoderm larvae can be

modified by acclimating adults to reduced salinity and that the ova are more strongly influenced by low salinity than spermatozoa.

The objectives of this research was to (1) examine the effects of salinity on the development, metabolic rates, and larval tolerances of five species of echinoderms which are found in waters of varying salinity (2) attempt to correlate the observed developmental rates and tolerances with known adult distributions along salinity gradients; and (3) determine if there is any reproductive advantage or potential fitness gain in acclimating adults to low salinity waters. The species chosen for investigation were: the green sea urchin, Strongylocentrotus droebachiensis; the purple sea urchin, Strongylocentrotus purpuratus; the white sea urchin, Strongylocentrotus pallidus; the seastar, Pisaster ochraceus; and the Florida urchin, Lytechinus variegatus. This work will further our understanding of echinoderm biology by yielding information concerning larval salinity tolerances and their effect on adult distribution along salinity gradients.

This dissertation is divided into 2 chapters. Chapter one is a reprint of a paper published in the Canadian Journal of Zoology (Vol 63, pp. 1531-1538) entitled "Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms". Chapter 2 is a manuscript entitled "Does salinity acclimation affect the larval tolerance, physiology, and early development of Strongylocentrotus droebachiensis (O.F.

Muller, 1776), S. pallidus (G.O. Sars, 1871), and Lytechinus
variegatus (Lamarck) (Echinodermata: Echinoidea)?"

CHAPTER 1

EFFECTS OF SALINITY ON LARVAL TOLERANCE AND EARLY
DEVELOPMENTAL RATES OF FOUR SPECIES OF ECHINODERMS

Reprinted from: Canadian Journal of Zoology 63(7):1531-1538 (1985)

Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms

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ROLLER, R. A., and W. B. STICKLE. 1985. Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms. *Can. J. Zool.* 63: 1531–1538.

Larval tolerances and developmental rates of four species and one hybrid cross of echinoderms were determined as a function of salinity. Fertilization and development of *Strongylocentrotus droebachiensis* (O.F. Muller, 1776), *Strongylocentrotus purpuratus* (Stimpson, 1857), *Strongylocentrotus pallidus* (G.O. Sars, 1871), and *Pisaster ochraceus* (Brandt, 1835) larvae were observed. A hybrid was created by fertilizing eggs of *S. droebachiensis* with sperm from *S. pallidus*. Fertilized eggs were placed in filtered, natural seawater (30‰ S) diluted to 30, 27.5, 25, 22.5, and 20‰ S and cleavage and larval development were observed over 32 days. Developmental rates differed among salinity treatments within each species. For each species, embryos and larvae at lower salinities (20, 22.5, and 25‰ S) tended to develop more slowly than those at higher salinities (27.5 and 30‰ S). *Pisaster ochraceus* and *S. droebachiensis* larvae survived all salinity treatments throughout the experimental period. The hybrid larvae exhibited salinity tolerances and developmental rates intermediate between values reported for the more stenohaline larvae of *S. pallidus* and those for the more euryhaline larvae of *S. droebachiensis*. The interaction of a secondary stressor (food availability) with salinity was examined; algal concentration did not influence the survival, developmental rate, or length of *P. ochraceus* or *S. droebachiensis* larvae.

ROLLER, R. A., et W. B. STICKLE. 1985. Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms. *Can. J. Zool.* 63: 1531–1538.

La tolérance larvaire à la salinité et le taux de développement en fonction de la salinité ont fait l'objet d'une étude chez quatre espèces et un hybride d'échinodermes. La fécondation et le développement ont été étudiés chez des larves de *Strongylocentrotus droebachiensis* (O.F. Muller, 1776), de *Strongylocentrotus purpuratus* (Stimpson, 1857), de *Strongylocentrotus pallidus* (G.O. Sars, 1871), et de *Pisaster ochraceus* (Brandt, 1835). La fécondation d'œufs de *S. droebachiensis* par du sperme de *S. pallidus* a donné lieu à un hybride. Les œufs fécondés ont été placés dans de l'eau de mer naturelle filtrée (30‰) diluée à 30, 27,5, 25, 22,5 et 20‰. La division et le développement larvaire ont ensuite été suivis durant 32 jours. La vitesse du développement larvaire variait en fonction de la salinité chez toutes les espèces: les embryons et les larves avaient tendance à se développer moins vite aux salinités les plus faibles (20, 22,5 et 25‰) qu'aux salinités les plus fortes (27,5 et 30‰). Les larves de *P. ochraceus* et de *S. droebachiensis* ont survécu durant toute l'expérience à toutes les salinités. Les larves hybrides ont manifesté une tolérance à la salinité et un taux de développement intermédiaires entre les valeurs obtenues chez les larves plus sténohalines de *S. pallidus* et les larves plus euryhalines de *S. droebachiensis*. L'addition d'un facteur de stress secondaire (la disponibilité de la nourriture) a révélé que la concentration des algues n'influence ni la survie, ni le taux de développement ou la longueur des larves de *P. ochraceus* ou de *S. droebachiensis*.

[Traduit par le journal]

Introduction

Although the phylum Echinodermata is well-known as an exclusively marine assemblage, populations of a number of species have been found in brackish water habitats (Binyon 1966, 1972; Thomas 1961; Stickle and Denoux 1976; Stancyk and Shaffer 1977; Turner and Meyer 1980; Pagett 1978; Himmelmann *et al.* 1983). The distribution of echinoderms along salinity gradients is believed to be limited because they possess a permeable body wall and lack differentiated osmoregulatory and excretory organs (Binyon 1966, 1972). In addition, within the zone of tolerance, the energy budget of *Leptasterias hexactis* was found to vary directly with environmental salinity (S) so that an average-weight starfish accrued 14.7 cal/day (1 cal = 4.1868 J) at 30‰ S and 2.9 cal/day at 20‰ S but lost 10.7 cal/day at 15‰ S (Shirley and Stickle 1982a, 1982b).

Stickle and Denoux (1976) have shown that populations of four species of echinoderms from the glacier influenced region of the Lynn Canal in southeastern Alaska tolerated fluctuating salinity during the summer months when the amplitude of salinity variation ranged from 11.4 to 17.2‰ during semi-diurnal tidal cycles. Comparisons of the salinity tolerance of the benthic stage of three species of echinoderms from the Alaskan populations (Sabourin and Stickle 1981; Shirley and

Stickle 1982a) with that of populations of those species from the high salinity environment near Friday Harbor, WA have shown that the populations of *Eupentacta quinquesemita* and *Leptasterias hexactis* from the low salinity environment are more tolerant of low salinity than populations from Friday Harbor (Stickle *et al.*). No significant difference existed between the salinity tolerances of the two populations of benthic *Strongylocentrotus droebachiensis*.

Several investigators have shown echinoderm larvae to be particularly sensitive to low salinity (Thorson 1946; Fenchel 1965; Binyon 1972; Watts *et al.* 1982). This project was designed to determine the effects of salinity on the tolerance and developmental rates of one species of asteroid (*Pisaster ochraceus* (Brandt, 1835)), three species of echinoids (*Strongylocentrotus droebachiensis* (O. F. Muller, 1776), *Strongylocentrotus purpuratus* (Stimpson, 1857), and *Strongylocentrotus pallidus* (G. O. Sars, 1871)), and one hybrid (*S. droebachiensis* (♀) × *S. pallidus* (♂)) from Friday Harbor, WA, a high-salinity environment. The rationale for using the hybrid cross is to attempt to determine the role that a species'

¹Stickle, W. B., M. Rotenberry, and D. Foltz. Population differences in the salinity tolerance, activity coefficients, and genetic composition of three species of echinoderms. Submitted for publication.

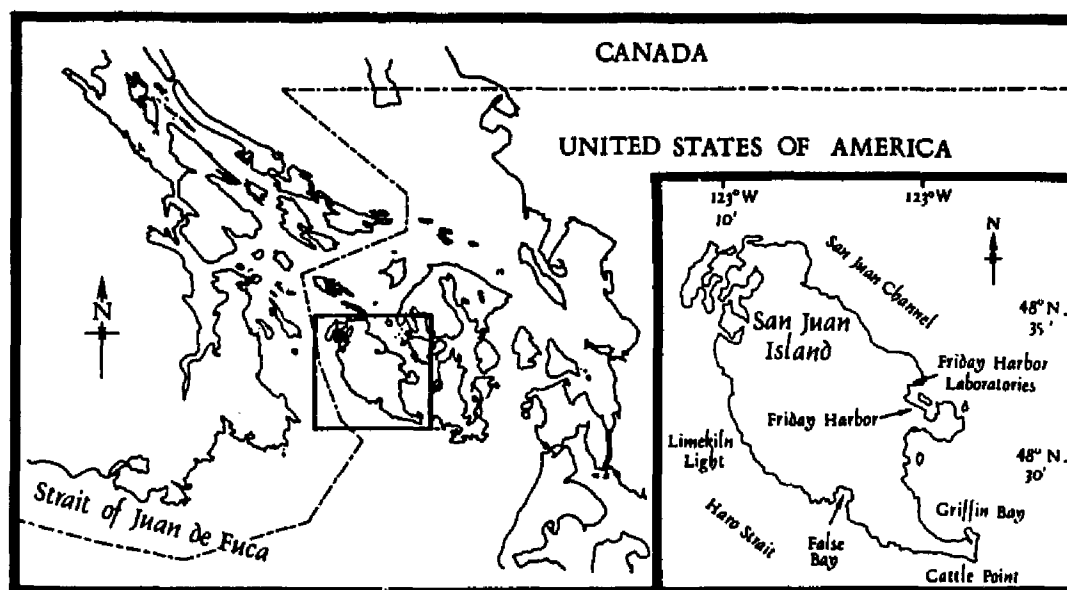


FIG. 1. San Juan Archipelago; inset, collection site on San Juan Island.

genetic makeup plays in the adaptation to salinity gradients. The determination of the salinity tolerance and growth of both the larval and benthic stages in the life cycle of these echinoderms will provide insight into the selective forces that salinity gradients place on these species of echinoderms.

Materials and methods

Mature adults of four echinoderm species were collected from San Juan Island, WA (U.S.A.) during May 1984 (Fig. 1). Adult *Pisaster ochraceus* and *Strongylocentrotus purpuratus* were collected during low tide at Lime Kiln Light on the west side of San Juan Island. *Strongylocentrotus droebachiensis* was collected during low tide at Cattle Point, on the southwestern tip of San Juan Island. *Strongylocentrotus pallidus* was collected at 70–90 m depth during a dredging trip on the R/V *Nugget* in San Juan channel. All specimens were returned to the Friday Harbor laboratories and maintained in a running seawater system.

Water temperature and salinity measurements in the seawater system were taken daily and both showed slight variation during the 32-day experimental period. Water temperature ranged from 9.0 to 11.0°C ($\bar{x} \pm SE = 9.9 \pm 0.10^\circ\text{C}$; $n = 32$) and salinity ranged from 30.0 to 32‰ S ($30.5 \pm 0.1\%$ S ; $n = 32$). Little seasonal variation occurs in the salinity of the waters surrounding San Juan Island. In a 5-year study of seasonal variations in surface temperature and salinity at the Friday Harbor laboratory, Phifer and Thompson (1937) found mean monthly salinity to vary from 29.7‰ in July to 30.7‰ in October. Furthermore, the water mass was nearly homogenous in salinity to a depth of 100 m.

Male and female *S. droebachiensis*, *S. purpuratus*, and *S. pallidus* were induced to spawn by coelomic injection with approximately 2 mL of 0.5 M KCl (Strathmann 1974). Female *P. ochraceus* were dissected, and ovarian maturation was induced by immersion in a 10^{-6} M solution of 1-methyladenine in seawater (Stevens 1970; Strathmann and Vedder 1977). All eggs were washed in filtered seawater and were fertilized with sperm from males of the same species. In the case of *P. ochraceus*, the testes were dissected from the adult to obtain sperm. A hybrid cross was created by fertilizing eggs of *S. droebachiensis*

with sperm from *S. pallidus* (Strathmann 1981). The percent fertilization for each species was as follows: *S. droebachiensis*, 98%; *S. purpuratus*, 91%; *S. pallidus*, 90%; hybrid, 88%; *P. ochraceus*, 94%. The criteria used in determining the success of fertilization were the formation of the fertilization envelope and the first cleavage of the zygote.

Two hundred fertilized eggs from each species were placed in 200 mL of filtered (1.0 μm) seawater at each of the following salinities: 30, 27.5, 25, 22.5, and 20‰ (temperature was $9.9 \pm 0.10^\circ\text{C}$ ($\bar{x} \pm SE$); $n = 32$). Developing embryos and larvae were maintained at these salinities throughout the experimental period. The water in each culture was changed daily during the course of the experiment. When the bipinnaria or pluteus stage was reached the larvae were fed *Dunaliella tertiolecta* daily.

During the early cleavage stages the cultures were examined hourly to determine developmental rates. After the swimming larval stage (bipinnaria or pluteus) was reached, the cultures were examined daily. A sample of 10 embryos or larvae was examined for the determination of developmental rates. Since mortality of all of the embryos or larvae in a culture of a particular salinity occurred within a very narrow time span (hours), we were unable to obtain an estimate of percent survival for any culture to a given stage. Survival was instead expressed as the number of days to which the embryos or larvae of a particular culture survived after fertilization. Length and width measurements (Fig. 2) were made on all embryos and larvae at each examination with an ocular micrometer. For echinoplutei, two length measurements were made: total length (L) was the distance from the posterior tip of the pluteus to the tip of the postoral arms; and midline length (L') was the distance from the posterior tip to the postoral transverse ciliated band. Width measurements for the plutei were taken at the most posterior extent of the ciliated band, dorsal to the postoral arms.

The interaction of a secondary stressor (low food availability) with salinity was examined for *P. ochraceus* by first dividing the cultures in half. One-half of the bipinnariae of *P. ochraceus* were fed a relatively high concentration of *D. tertiolecta* (6000 cells/mL) which permits maximum ingestion by (Strathmann 1971) and maximum growth of (Lucas 1982) some asteroid larvae. The other half of each *P. ochraceus* culture was fed a concentration of algae (500 cells/mL)

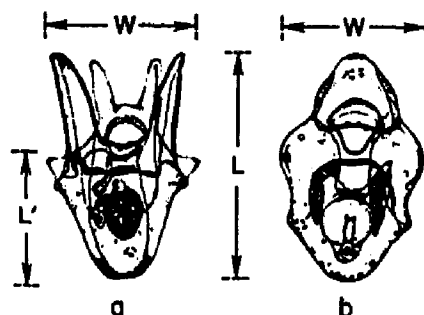


FIG. 2. Diagram illustrating measurements of (a) plutei of *Strongylocentrotus droebachiensis* and (b) bipinnaria of *Pisaster ochraceus*. L, total length of larvae; L', midline length; W, width of larvae.

well below the saturation concentration. This experiment was repeated for the *S. droebachiensis* cultures. Two-way analysis of variance (ANOVA) (Anonymous 1982) was used to determine significant variation among treatments, with tests of salinity and algal concentration main effects as well as their interaction. Scheffe's test (Snedecor and Cochran 1980) was used to identify which treatments were significantly different from each other in the larval size experiments as a function of salinity and algal ration. Regression analyses were conducted using a general linear model (GLM) procedure (Anonymous 1982). Significance is reported for the *F*-test of model versus error sums of squares in this analysis of regression. The coefficient of determination (r^2) values (Steel and Torrie 1980) are from the fit of these linear regression models.

Results

Survival

Considerable variation exists in the salinity tolerance of the four species of echinoderm larvae, and the hybrid cross (Table 1). The larvae of *S. droebachiensis* and *P. ochraceus* survived the 32-day exposure at all experimental salinities; however, the embryos and bipinnariae of *P. ochraceus* at 20‰ S were very abnormal by the 32nd day compared with those at higher salinities (Figs. 3–6). The plutei of *S. droebachiensis* maintained at all of the experimental salinities were normal in appearance (Figs. 7 and 8).

For *S. pallidus* and *S. purpuratus* only larvae maintained at 27.5 and 30‰ S survived the 32-day experimental period. The length of survival of larvae increased with increasing environmental salinity for both species. The hybrid plutei were normal in appearance at all salinities above and including 22.5‰ (Fig. 9). The survival data for *S. purpuratus* and *S. pallidus* at 20, 22.5, and 25‰ S represent conservative estimates because there were many deformed larvae still moving during the early days of the experimental period. Hybrid larvae from the *S. droebachiensis* (♀) – *S. pallidus* (♂) cross exhibited salinity tolerances that were intermediate between those of the two species.

Developmental rates

Developmental rates increased directly with increasing salinity for larvae of all four echinoderm species and the hybrid cross. The developmental rate of *P. ochraceus* larvae was slower at 20, 22.5, and 25‰ S in comparison with larvae cultured at 27.5 and 30‰ S (Table 2). The presence of abnormal gastrulae at 20‰ S after 4 days exposure suggests that larvae cultured at 22.5 and 25‰ S may also not develop nor-

TABLE 1. Survival time (days) as a function of salinity

Species	Salinity (‰)				
	20	22.5	25	27.5	30
<i>P. ochraceus</i>	>32*	>32	>32	>32	>32
<i>S. droebachiensis</i>	>32	>32	>32	>32	>32
Hybrid	2	>32	>32	>32	>32
<i>S. pallidus</i>	1.5	1.5	7	>32	>32
<i>S. purpuratus</i>	2	2.5	9	>32	>32

*Abnormal larvae.

TABLE 2. Salinity influence on developmental rates for *Pisaster ochraceus* are given as elapsed time to each developmental stage

Stage	Salinity (‰)				
	20	22.5	25	27.5	30
Two polar bodies	2 h	2 h	2 h	2 h	2 h
8–16 cell	15 h	15 h	12 h	12 h	12 h
Unhatched					
blastula	49 h	40 h	36 h	18 h	18 h
Hatching	70 h	48 h	42 h	36 h	36 h
Gastrula*	96 h	62 h	59 h	49 h	49 h
Bipinnaria†					
with hydrocoel	‡	10 days	7 days	6 days	6 days

*Body length/archenteron length is 3/1.

†640 µm in total length.

‡Abnormal larvae, 400 µm in length.

mally because of slower developmental rates relative to the developmental rates of larvae at 27.5 and 30‰ S.

The developmental rate of the echinoid *S. droebachiensis* showed no evidence of a salinity effect through the prism stage but development to the four-arm pluteus stage was slowed in larvae cultured at 20 and 22.5‰ S (Table 3). In contrast there was a marked effect of salinity on the developmental rate of *S. pallidus* during the early stages of development (Table 4). Furthermore, delayed development of the early stages of *S. pallidus* was correlated with larval mortality at those salinities during later developmental stages (Table 1).

The developmental rates of *S. droebachiensis* (♀) – *S. pallidus* (♂) hybrid larvae were intermediate between the developmental rates of the parental species (Table 5). Normal developmental times were lengthened at 22.5‰ for the hybrid, compared with developmental times at 25, 27.5, and 30‰ S for *S. droebachiensis* but the hybrid developed normally at a salinity (22.5‰) that was 5‰ lower than the salinity at which *S. pallidus* developed normally.

The developmental rate of *S. purpuratus* larvae was greatly lengthened at 20, 22.5, and 25‰ S compared with rates at 27.5 and 30‰ S (Table 6). Furthermore, slower developmental rates were correlated with eventual mortality of larvae at 20, 22.5, and 25‰ S (Table 1).

Algal concentration

Algal concentration had no effect on larval size of either *P. ochraceus* or *S. droebachiensis* according to a two-way ANOVA with algal concentration and salinity as main effects. There were, however, significant effects of salinity on the larval size of both species. Since larval length and width measurements of both species yielded the same pattern when measurements were made on day 30 (Table 7) and no signifi-

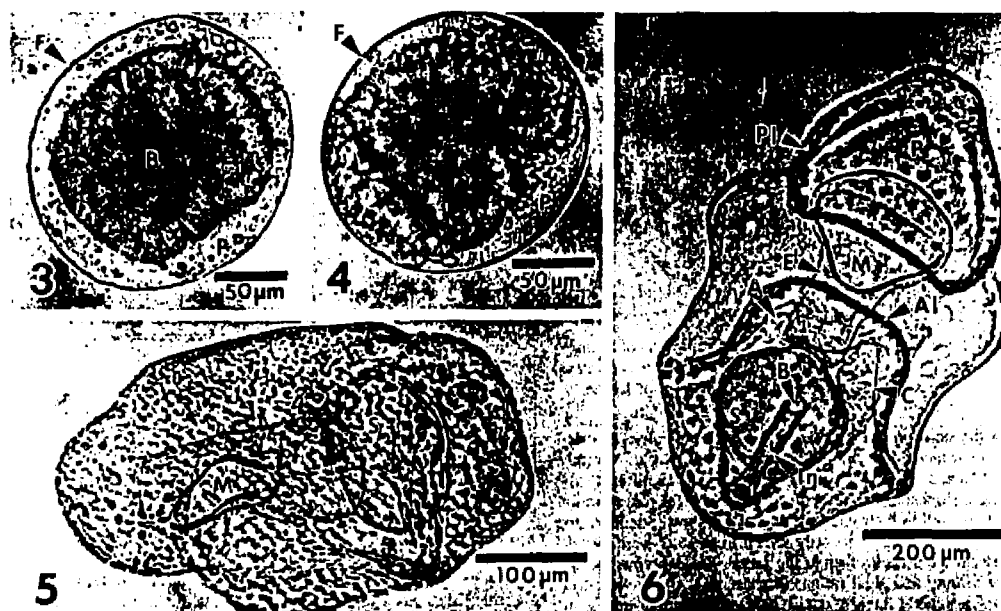


FIG. 3. Normal blastula of *Pisaster ochraceus* cultured in 30‰ S seawater (18 h postfertilization). F, fertilization envelope; P, perivitelline space; B, blastocoel; arrows indicate margin of blastocoel. FIG. 4. Abnormal blastula of *Pisaster ochraceus* cultured in 20‰ S seawater (49 h postfertilization). F, fertilization envelope; P, perivitelline space; arrow, malformed cells. FIG. 5. Ventrolateral view of abnormal *Pisaster ochraceus* bipinnaria in 20‰ S seawater (2 weeks postfertilization). M, mouth; S, stomach. Note absence of preoral and anal loop ciliation. FIG. 6. Ventral view of normal bipinnaria of *Pisaster ochraceus* in 30‰ S seawater (6 days postfertilization). A, anal lobe; AI, anal loop cilia; B, blastopore (anus); C, left coelom; E, esophagus; In, intestine; M, mouth; P, preoral lobe; PI, preoral loop ciliation; S, stomach.

TABLE 3. Salinity influence on developmental rates for *Strongylocentrotus droebachiensis* is given as elapsed time to each stage

Stage	Salinity (‰)				
	20	22.5	25	27.5	30
8 cell	7 h	7 h	6 h	6 h	6 h
16 cell	8 h	8 h	7 h	7 h	7 h
Unhatched blastula	18 h	18 h	18 h	18 h	18 h
Prism	3 days	3 days	3 days	3 days	3 days
Four arm* pluteus	7 days	7 days	6 days	5 days	5 days
Four arm† pluteus	9 days	9 days	7 days	7 days	7 days

*Total length is 500 µm.

†Vestibule present.

cant differences existed between larvae maintained at *Dunaliella tertiolecta* concentrations of 500 or 6000 cells/mL, length measurements are given for both species at 6000 algal cells/mL only (Fig. 10). The larval length of both species fed 6000 cells/mL varied directly with ambient salinity as evidenced by significant (r^2) linear regressions of larval length on salinity, and larvae maintained at 20‰ S were significantly smaller than those maintained at all higher salinities (Scheffe's test). The regression equation for *P. ochraceus* is given as length (µm) = $-105 + (\text{salinity} \times 30.24)$ ($r^2 = 0.736$; $n = 50$; $p < 0.001$) and the equation for *S. droebachiensis* is length

TABLE 4. Salinity influence on developmental rates for *Strongylocentrotus pallidus* is given as elapsed time to each stage

Stage	Salinity (‰)				
	20	22.5	25	27.5	30
8 cell	10 h	6 h	6 h	6 h	6 h
Unhatched blastula	36 h	36 h	20 h	20 h	20 h
Gastrula*	—†	—	48 h	45 h	45 h
Prism	—	—	4 days	3 days	3 days
Four arm‡ pluteus	—	—	—	6 days	6 days

*Body length/archenteron length is 2/1.

†—, dead culture.

‡Total body length is 500 µm.

(µm) = $244 + (\text{salinity} \times 14.97)$ ($r^2 = 0.601$; $n = 50$; $p < 0.001$). As indicated earlier many of the starfish bipinnariae cultured at 20‰ S were abnormal and eventually died.

Discussion

The pelagic periods of all four species of echinoderm studied suggest the potential for extensive dispersal in nature (Strathmann 1978). The short-term stenohalinity of the larval stages of *S. pallidus* and *S. purpuratus* likely limits those species to high salinity waters whereas salinity as low as 20‰ S does not appear to be as great a selective force affecting the distribution of the larval stages of *P. ochraceus* or *S. droe-*

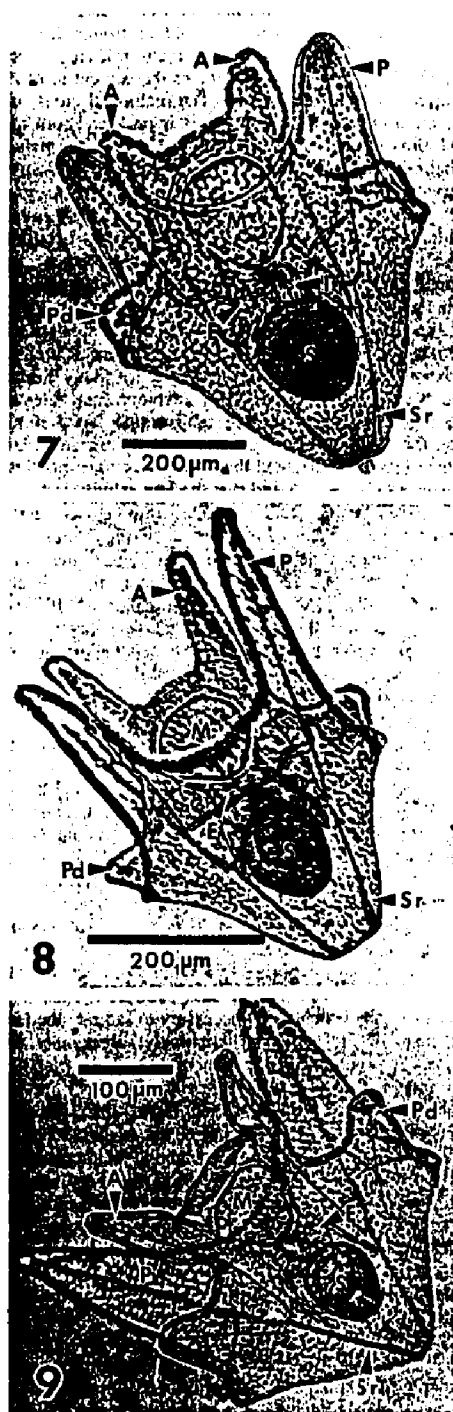


TABLE 5. Salinity influence on developmental rates for hybrid between a ♀ *S. droebachiensis* and a ♂ *S. pallidus* is given as elapsed time to each developmental stage

Stage	Salinity (‰)				
	20	22.5	25	27.5	30
16 cell	20 h	7 h	7 h	7 h	7 h
Unhatched blastula	—*	24 h	20 h	20 h	20 h
Gastrula†	—	26 h	24 h	24 h	24 h
Prism	—	4 days	3 days	3 days	3 days
Four arm pluteus‡	—	6 days	4 days	4 days	4 days

*—, dead culture.

†Body length/archenteron length is 2/1.

‡Total body length is 400 μm.

TABLE 6. Salinity influence on developmental rates for *Strongylocentrotus purpuratus* is given as elapsed time to each stage

Stage	Salinity (‰)				
	20	22.5	25	27.5	30
16 cell	20 h	10 h	6 h	6 h	6 h
Unhatched blastula	—*	—	20 h	20 h	20 h
Prism	—	—	6 days	3 days	3 days
Four arm pluteus	—	—	8 days†	5 days	5 days
Four arm‡ pluteus	—	—	—	8 days	8 days

*—, dead culture.

†Very deformed but still living larvae.

‡Vestibule present.

bachiensis.

Although *Pisaster ochraceus* larvae survived the entire salinity range to which they were exposed, larval developmental rates were slower at 20 and 22.5‰ S and bipinnaria were abnormal in appearance and smaller at 20‰ S, which suggests that they would eventually die. Less than half of the pelagic larval period, found by Strathmann (1978) to last between 76 and 228 days, had elapsed by the end of the 32-day experimental period. The dominant carnivore on the lower rocky intertidal community of the outer Pacific coast of North America is *P. ochraceus* (Paine 1966), found from Sitka, AK to Cedros Island, Baja California (Lambert 1981; Morris *et al.* 1980). In the inside waters of the British Columbia coast, and in lower Puget Sound, *Pisaster* is replaced by *Evasterias troschelii* (Lambert 1981). Fifty-seven *P. ochraceus* were

FIG. 7. Ventral view of normal pluteus of *Strongylocentrotus droebachiensis* in 30‰ S seawater (5 days postfertilization). A, anterolateral arms; E, esophagus; In, intestine; M, mouth; P, postoral arms; Pd, posterodorsal arms; S, stomach; Sr, skeletal rods. FIG. 8. Ventral view of normal pluteus of *Strongylocentrotus droebachiensis* in 20‰ S seawater (7 days postfertilization). A, anterolateral arms; E, esophagus; M, mouth; P, postoral arms; Pd, posterodorsal arms; S, stomach; Sr, skeletal rods. FIG. 9. Dorsal view of healthy hybrid (*S. droebachiensis* ♀ × *S. pallidus* ♂) pluteus in 22.5‰ S seawater (6 days postfertilization). A, anterolateral arms; E, esophagus; M, mouth; P, postoral arms; Pd, posterodorsal arms; S, stomach; Sr, skeletal rods.

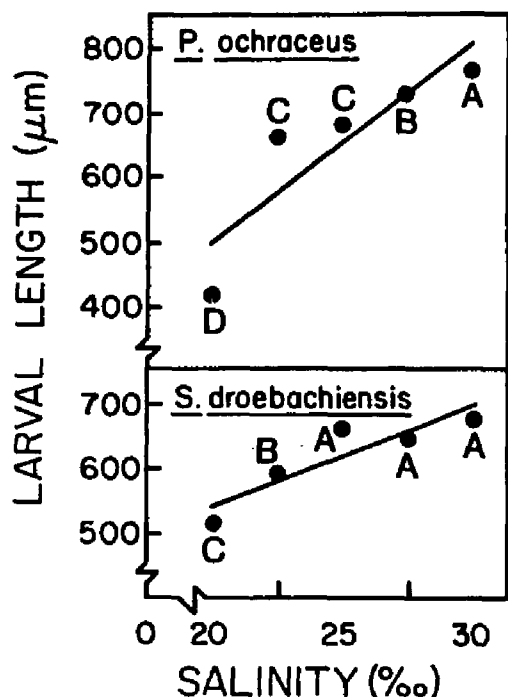


FIG. 10. Length of *Pisaster ochraceus* bipinnaria and *Strongylocentrotus droebachiensis* plutei after 30 days exposure at environmental salinities of 20, 22.5, 25, 27.5, and 30‰ in the presence of 6.0×10^3 *Dunaliella tertiolecta* per millilitre. Mean lengths ($N = 10$) are plotted for each salinity and letters near each data point indicate Scheffe's groupings; data points sharing the same letter are not significantly different from each other. Regression of larval length as a function of salinity is also plotted.

recovered two summers after 87 adults from a high-salinity habitat (Prince William Sound, south-central Alaska) were transplanted to a low-salinity (as low as 12.5‰) habitat (Auke Bay, AK) (W. B. Stickle and C. E. O'Clair, personal observation). No juveniles were recovered, however, which suggests that either *P. ochraceus* are incapable of reproducing at that location or the larval stages will not tolerate reduced salinity.

Larval survival of *S. droebachiensis* was unaffected by 32 days exposure to the entire 20–30‰ S range of our experimental design but normal development to the four-arm pluteus stage was slower at 20 and 22.5‰ S than at 25, 27.5, and 30‰ S and there was a direct relationship between larval size at 30 days and salinity. The planktonic larval stage of the green sea urchin cultured at 30‰ S at Friday Harbor varies from 51 to 152 days (Strathmann 1978). The habitat of the benthic stage of *S. droebachiensis* is circumpolar and green sea urchins from both the low and high salinity environments near Juneau, AK and Friday Harbor tolerate environmental salinities as low as 13‰ S (Sabourin and Stickle 1981; Stickle *et al.* see footnote 1).

The hybrid-cross experiment between a female *S. droebachiensis* and a male *S. pallidus* supports the contention that there is a genetic component to the euryhalinity of *S. droebachiensis*. Hybrid larvae exhibited survival and develop-

mental rate patterns intermediate between those of the two parental species. The conclusion would be stronger if the same observations had been made for the reciprocal cross (paternal *S. droebachiensis*) but with this cross the percent fertilization is near zero (Strathmann 1981). The reciprocal cross, if feasible, and backcrosses between the F_1 hybrids and both species would allow further clarification of the genetic component of euryhalinity in *S. droebachiensis*.

Larval tolerance and developmental rates of *S. purpuratus* and *S. pallidus* correlate well with the distributional pattern of the adult stages. *Strongylocentrotus purpuratus* occurs from Alaska to Baja California, along the outer coast of British Columbia and Alaska (Ricketts and Calvin 1968) and is only encountered in the Puget Sound region on shores that are somewhat exposed to strong wave action such as the western side of San Juan Island (Kozloff 1983). Therefore, benthic urchins are not exposed to brackish water. Larvae are likely limited to salinities greater than 25‰ if slow developmental rates at 20, 22.5, and 25‰ are indicative of eventual larval mortality during the later stages of development. Giese and Farmanfarmaian (1963) determined that the 35-day tolerance limit of *S. purpuratus* is 29‰ S and that the low salinity larval tolerance is 28‰; their larval tolerance limit agrees very well with our value of 25–27.5‰ S. Likewise, *S. pallidus* are usually restricted to depths greater than 50 m in the San Juan Archipelago (Kozloff 1974) where ambient salinity is always greater than 30‰ (Thomson 1981; Collias *et al.* 1974). The results of this investigation indicate that the larval stages of both *S. purpuratus* and *S. pallidus* are stenohaline and limited to salinities greater than 27.5‰. Larval developmental times through settling at 30‰ S are considerably longer than the 32-day experimental period of this study: 63–86 days for *S. purpuratus* and 63 days for *S. pallidus* (Strathmann 1978). R. R. Strathmann (personal communication) has indicated that the species used in this investigation could possibly be reared in a shorter time than previously reported (Strathmann 1978).

Differences in food availability had no visible effect on the developmental rates, survival, or larval size of either *P. ochraceus* or *S. droebachiensis* as a function of salinity. Two possibilities exist for the lack of a statistical relationship between the algal ration of *Dunaliella tertiolecta* provided to *P. ochraceus* and *S. droebachiensis* and their larval length or width after 30 days exposure to a salinity gradient (Table 7). Both algal concentrations, 500 and 6000 cells/mL, may be higher than the concentration necessary to saturate the feeding mechanism of the larvae. Alternatively, algae may not be necessary to accomplish development through the first 30 days of the planktonic phase of the life cycle for either species.

The direct relationship between larval size and ambient salinity after 30 days exposure may be due to variation in either the rate of energy acquisition or metabolic maintenance costs. Stickle (1985) has found that variation in energy acquisition along gradients of environmental factors is much more important than variation in metabolic maintenance costs for five species of carnivorous marine invertebrates but we do not know if this finding is also valid for planktonic larvae. It is likely that the interaction between the availability of food and salinity would be important during later, critical stages of development such as settlement and metamorphosis.

The geographic area over which larvae are dispersed from a resident population can greatly influence the genetic composition of isolated populations of echinoderms. The duration of the planktonic phase of a species as well as larval behavioral

TABLE 7. Analysis of variance table of *Strongylocentrotus droebachiensis* and *Pisaster ochraceus* cultured at 20, 22.5, 25, 27.5, or 30‰ S for 32 days and provided *Dunaliella tertiolecta* at concentrations of 500 or 6000 cells/mL.

Species	Source	df	MS	F
<i>S. droebachiensis</i>	Total	99		
	Food	1	154/1232	0.08NS/0.91NS
	Salinity	4	98257/53310	51.93**/39.50**
	Food × salinity	4	618/1341	0.33NS/0.99NS
	Error	90	1892/1350	
<i>P. ochraceus</i>	Total	99		
	Food	1	615/1592	0.93NS/2.45NS
	Salinity	4	347982/144174	527.16**/221.72**
	Food × salinity	4	1601/1558	2.42NS/2.40NS
	Error	90	660/650	

NOTE: Larval length/width measurements were made as shown in Fig. 2 and given in micrometers. NS indicates nonsignificant effects at the $p \leq 0.05$ level; ** indicates $p < 0.001$.

characteristics influence the degree of dispersal and adaptation of isolated populations of a species to local environmental conditions (Burton and Feldman 1982). Echinoderm larvae are not thought to utilize behavioral patterns to maintain their position in a geographic area, as crustaceans do (Cronin and Forward 1982). Strathmann (1982) concluded on theoretical grounds that the large-scale spread of larvae from a given population is an accidental though important consequence of a long planktonic period, but the spread of offspring confers no short-term advantage to that population. Large-scale larval dispersion between populations if associated with increased genetic variability may, however, increase the frequency of polymorphic loci in each population and increase the physiological fitness of those individuals that contain a large number of heterozygous alleles at those loci (Garton 1984; Garton *et al.* 1984; Garton and Stickle 1985). Large-scale spread of *S. droebachiensis* larvae could account for the euryhalinity of a population of green sea urchins from the high salinity environment near Friday Harbor (Stickle *et al.*, see footnote 1). However, this process does not appear to be operative for the other echinoderm species studied in which local selection may limit the species to near full-strength salinity.

Experimental design strongly influences the results obtained with respect to salinity tolerance and development of echinoderm larvae along salinity gradients. The present experiment was designed to determine the tolerance and developmental capabilities of fertilized ova upon transfer from full-strength seawater (30‰ S), to which the parents were acclimated, to ambient salinities of 20, 22.5, 25, 27.5, and 30‰ S. Gezelius (1963) found that the cleavage rate of fertilized *Psammachinus miliaris* eggs at a given temperature depended on the salinity to which the parents had been adapted. Greenwood and Bennett (1981) performed osmotic shock experiments on the spermatazoa and ova of *Parechinus angulosus* by preincubating each at 45 temperature-salinity combinations before fertilization under conditions of temperature (15°C) and salinity (34‰ S) optimal for maximum fertilization. Temperature gradients had the greatest effect on spermatazoa but salinity gradients proved injurious to the ova. Giese and Farmanfarmanian (1963) found that the gastrulae of *Strongylocentrotus purpuratus* tolerated and differentiated in the same salinity range (28–38.5‰ S) as that tolerated by the eggs and adults. Watts *et al.* (1982) transferred 2-day-old type 1 and type 2 larvae of the asteroid *Echinaster* species complex to three tem-

peratures and three salinities. They found that salinity was the dominant factor affecting development and growth. Hybrids showed intermediate development and growth responses at the apparent optimal conditions. Hybrids also exhibited stronger maternal characteristics. Most larval mortality occurred after the mouth was formed. These studies indicate that the salinity tolerance and developmental rate of echinoderm larvae can be modified by acclimating adults to reduced salinity and that ova are more strongly influenced by low salinity than spermatazoa. Only properly designed experiments which control for the acclimation history and genetic composition of parents will allow for a completely ecologically meaningful characterization of larval tolerance and developmental rates along salinity gradients.

The stenohalinity of the pelagic larval stages of *Strongylocentrotus purpuratus* and *S. pallidus* likely limit the distributional patterns of these sea urchins. Our experiments are inconclusive with respect to the correlation between the effects of salinity on the larval tolerance and developmental rates of *P. ochraceus* and adult distributional patterns in estuarine waters. Larval survival and developmental rates are well correlated with the euryhalinity of *S. droebachiensis*. Future research will compare results obtained with populations adapted to high salinity to those of populations subjected in nature to fluctuating salinity which may have been influenced by local environmental selection.

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CHAPTER 2

DOES SALINITY ACCLIMATION AFFECT THE LARVAL TOLERANCE,
PHYSIOLOGY, AND EARLY DEVELOPMENT OF STRONGYLOCENTROTUS
DROEBACHIENSIS (O.F. MULLER, 1776), S. PALLIDUS (G.O. SARS,
1871), AND LYTECHINUS VARIEGATUS (LAMARCK) (ECHINODERMATA:
ECHINOIDEA)?

ABSTRACT

Larval tolerance and developmental rates of Strongylocentrotus droebachiensis, S. pallidus, and Lytechinus variegatus were determined for two experiments: 1) Direct transfer of fertilized eggs to lowered salinity; and 2) Acclimation of adult urchins to lowered salinity for 1-4 wk prior to spawning. Fertilized eggs of S. droebachiensis and S. pallidus were directly transferred to 30, 25, 20, 15, 12.5, and 10⁰/ooS at 10°C. Adult S. droebachiensis and S. pallidus were also acclimated to the same salinities for 2, 3, and 4 wk prior to spawning. Fertilized eggs of L. variegatus were directly transferred to 35, 30, 27.5, 25, 20, 15, and 10⁰/ooS at 18 and 23°C. Adult L. variegatus were acclimated to the same salinities and temperatures for 1, 2, and 4 wk prior to spawning. For the acclimation experiments, fertilized eggs were cultured at the same temperature and salinity as adults. Development and survival to metamorphosis were examined in all cultures. Developmental

rates and percent survival to metamorphosis varied directly with salinity for larvae of all species. An observed increase in the number of abnormal plutei and a decrease in survival to metamorphosis occurred at salinities below 30⁰/oo for S. pallidus. S. droebachiensis plutei survived to settlement and metamorphosis at salinities above 17.5⁰/ooS. For L. variegatus, survival to metamorphosis decreased at salinities below 35⁰/oo (Q₁₀ values for metamorphosis= 0.380-0.384). There were significant (ANOVA) temperature and salinity effects on metabolic rates of L. variegatus plutei. L. variegatus and S. pallidus larvae are stenohaline when compared to the larvae of S. droebachiensis. Coelomic cavity lactic acid levels of adult S. droebachiensis and S. pallidus acclimated to low salinity were significantly higher than controls at 30⁰/ooS but there was no histological evidence of altered gonadal tissue. Gonadal NPS values of S. droebachiensis and S. pallidus were significantly lower at 17.5⁰/ooS than at the higher salinities. Cell volumes of fertilized eggs of all species exhibited osmotic swelling when exposed to lowered salinity. The percent volume regulation of fertilized eggs of the three species varied indirectly with salinity. The anaerobic environment of the coelomic fluids in urchins acclimated to low salinity adversely affected the viability of gametes. LC₅₀ values (⁰/ooS), developmental rates, and survival to metamorphosis indicate that acclimation of adult urchins to lower salinity prior to spawning and fertilization does not enhance development or survival of embryos exposed to low

salinity. The stenohalinity of larval L. variegatus and S. pallidus may limit adult distributions along salinity gradients.

INTRODUCTION

Echinoderms are considered to be stenohaline organisms with respect to their distribution along salinity gradients primarily because they possess a permeable body wall (Drouin et al., 1985) and lack differentiated osmoregulatory and excretory organs (Binyon, 1966, 1972); however, several species have been found to be more euryhaline than previously believed (Binyon, 1961; Lawrence, 1975; Stickle and Denoux, 1976; Stancyk and Schaffer, 1977; Turner and Meyer, 1980; as reviewed by Stickle and Diehl, 1987). Binyon (1972) stated that the more euryhaline populations of echinoderms occur only in regions such as the Baltic and Black Seas, where the dilution of full strength salinity has been gradual, taking place over many geological epochs, rather than in those regions subject to seasonal and/or diurnal fluctuations of salinity. Recently however, populations of echinoderms have been found in habitats subjected to tidal and/or seasonal reductions of ambient salinity (Thomas, 1961; Stickle and Denoux, 1976; Stancyk and Shaffer, 1977; Pagett, 1978; Turner and Meyer, 1980; Himmelman et al., 1983).

Populations of four species of echinoderms from the glacier influenced region of the Lynn Canal in southeastern Alaska tolerated fluctuating salinity during the summer months when the

amplitude of the salinity range varied from 11.4 to 17.2‰ over the course of semidiurnal tides (Stickle and Denoux, 1976). It was also demonstrated that populations of Eupentacta quinquesemita and Lepasterias hexactis from low salinity habitats are more tolerant of low salinity exposure than are populations of the same species from normally high salinity waters; however, no significant difference exists between the salinity tolerances of populations of S. droebachiensis from high and low salinity habitats (Sabourin and Stickle, 1981; Shirley and Stickle, 1982; Stickle and Diehl, 1987).

The developmental patterns of many marine invertebrates are known to be influenced by variations in salinity (MacInnes and Calabrese, 1979; Lucas and Costlow, 1979; Johns, 1981a, 1981b, 1982; Laughlin, 1983; Roller and Stickle, 1987). Several species of echinoderm larvae have also been shown to be particularly sensitive to fluctuating salinity (Binyon, 1972; Petersen and Almeida, 1976; Greenwood and Bennett, 1981; Watts et al., 1982; Drouin et al., 1985; Roller and Stickle, 1985).

The objectives of the present investigation were to (1) compare the effects of lowered salinity on the developmental patterns and survival to metamorphosis of larval Strongylocentrotus droebachiensis (O.F. Muller, 1776), Strongylocentrotus pallidus (G.O. Sars, 1871) and Lytechinus variegatus (Lamarck); (2) identify any possible benefits to the development or survival of larvae incurred by acclimating adult urchins to lowered salinity prior to spawning;

(3) determine if salinity and temperature variations affect the metabolic rates of larval *L. variegatus*.

MATERIALS AND METHODS

DIRECT TRANSFER STUDIES

Mature adults of Strongylocentrotus droebachiensis and S. pallidus were collected in the vicinity of San Juan Island, WA (U.S.A.) during February 1986. Specimens were collected at 70-90 m depth during a dredging trip on the R/V Nugget in San Juan Channel (48°35'N 123°W). All urchins were returned to the Friday Harbor Laboratories and maintained in a running seawater system. Water temperature and salinity of the seawater system were measured daily and both showed slight variation during the experimental period. Water temperature varied from 9.0 to 10°C ($\bar{x} \pm \text{S.E.} = 9.9 \pm 0.20^\circ\text{C}$; $n = 45$) and salinity ranged from 30.0 to 31.0‰ ($30.3 \pm 0.8^\circ\text{‰S}$; $n = 45$) during the course of the experiment. Little seasonal variation occurs in the salinity of the water surrounding San Juan Island. Phifer and Thompson (1937) found mean monthly salinity of waters in this area to vary from 29.7‰ in July to 30.7‰ in October. Furthermore the water mass was nearly homogenous in salinity to a depth of 100 m.

For the direct transfer experiments, male and female S. droebachiensis and S. pallidus were induced to spawn by coelomic injection with 2 ml of 0.5 M KCl (Strathmann, 1974). All eggs were washed in filtered (1.0 μm) seawater and were fertilized with sperm from males of the same species. The criteria used in determining the success of fertilization were the formation of the fertilization envelope and the first cleavage of the zygote.

Two hundred fertilized eggs from each species were placed in 200 ml of filtered (1.0 μ m) seawater at each of the following salinities: 30, 25, 20, 15, 12.5, and 10 $^{\circ}$ /oo (temperature was $9.9 \pm 0.20^{\circ}\text{C}$ ($\bar{x} \pm \text{S.E.}$; $n = 65$). Developing embryos and larvae were maintained at these salinities throughout the experimental period. Culture bowls were maintained in a plexiglass water table connected to the flow-through natural seawater system of the laboratories. The water in each culture was changed daily throughout the course of the experiment.

Mature Lytechinus variegatus were collected at St. Joseph Bay, Florida, U.S.A. (29 $^{\circ}$ 53'N; 85 $^{\circ}$ 24'W) during July 1985 and May 1986. All urchins were held overnight in a flow-through seawater tank before being transported to Louisiana State University. Adults were held in 38-l aquaria (10 urchins/aquarium) at the same temperature and salinity as the collection site for two days prior to spawning. Spawning of adults, and fertilization of eggs were by the same methods used for S. droebachiensis and S. pallidus.

Two hundred fertilized L. variegatus eggs were placed in 200 ml of filtered (1.0 μ m) seawater at either 18 or 23 $^{\circ}\text{C}$ and at each of the following salinities: 10, 15, 20, 25, 27.5, 30 and 35 $^{\circ}$ /oo. Culture bowls were maintained in an insulated, flow-through plexiglass water table connected to a temperature controlled water recirculator. Developing embryos and larvae were maintained under these conditions throughout the experimental period. The water in each culture was changed daily throughout

the course of the experiment.

Cell volume measurements were made on fertilized eggs of S. droebachiensis and S. pallidus 4 h after transfer to lowered salinity, and on L. variegatus eggs 3 h after transfer to low salinity. Controls consisted of fertilized eggs from the same salinity to which the adults were acclimated. Perfect osmometer calculations were made by dividing the highest salinity used for each species by the lower salinities. Cell volume ratios were also calculated to yield water dilution information for ninhydrin-positive substance concentrations and percent volume regulation information. The ratios were obtained by dividing the cell volume at 25, 20, and 17.5‰ by the cell volume at 30‰ for both S. droebachiensis and S. pallidus and by dividing the cell volume at 30, 27.5, 25, and 20‰ by the cell volume at 35‰ for L. variegatus. Percent volume regulation measurements were made by first calculating the difference between the perfect osmometer values and the cell volume ratios; The resultant values were then divided by the difference between the perfect osmometer value and the high salinity reference.

During the early cleavage stages all cultures were examined hourly to determine developmental rates. Once the pluteus stage was reached, the cultures were examined daily. Q_{10} values were calculated for metamorphosis of L. variegatus plutei at each salinity. All plutei were fed Dunaliella tertiolecta daily. Culturing of algae was by the method of Guillard (1975).

Percent survival to settlement and metamorphosis was determined for each culture.

The percent synchrony of early cleavage was determined for the L. variegatus cultures at each salinity 4 days after fertilization. Synchrony was determined by counting the number of embryos at the most prevalent developmental stage and then dividing by the total number of embryos in the culture.

Four days after settlement and metamorphosis 1200 juvenile L. variegatus each from the 30 and 35⁰/ooS at 23⁰C were back-transferred to 10, 15, 20, 25, 27.5, 30, and 35⁰/ooS (200 urchins/salinity). Percent survival after four days exposure was determined on duplicate samples from each salinity. Juveniles were fed a modification of the artificial diet of Klinger et al. (1986). The diet consisted of: a 3% agar matrix containing 5% dried food. The dried food consisted of: 29% shrimp, 19% sea trout, 33% seaweed, 17% wheat gluten, 1% lecithin, 0.5% vitamin C, and 0.5% vitamin B.

ACCLIMATION STUDIES

For the acclimation experiments, 12 adults of S. droebachiensis and S. pallidus were stepwise acclimated to 25, 20, 17.5, 15 and 10⁰/ooS (5⁰/oo per day) and held at each salinity for 2, 3, and 4 wk prior to spawning. After the desired acclimation was completed, spawning and fertilization of gametes was attempted. Upon successful fertilization, the embryos and larvae were reared at the salinity at which the parents were

acclimated.

In a second set of experiments, adult L. variegatus were collected from the same site off Florida in July of 1986. Adults were transported to the laboratory and placed into 38-l aquaria (10 urchins/aquarium) at the same temperature and salinity as the collection site. Each aquarium was connected via a flow-through apparatus to an additional 50-l seawater reservoir. The urchins were step-wise acclimated to the following salinities: 10, 15, 20, 25, 27.5, 30 and 35‰ at 23°C. Salinity was adjusted 5‰ per day and temperature was adjusted 2°C per day until the desired final values were attained. The urchins were allowed to acclimate to each salinity/temperature combination for 1, 2 or 4 weeks. After the desired acclimation time was completed, spawning and fertilization of gametes was attempted. Upon successful fertilization, the embryos and larvae were reared at the temperature and salinity at which the parents were acclimated.

Cell volume measurements, developmental rates, and survival to metamorphosis were determined as above for all species examined.

Ninhydrin-positive substances (NPS) were measured in the gonadal tissue of 2, 3, and 4 wk acclimated female S. droebachiensis and S. pallidus. Gonads were excised, then frozen in liquid nitrogen and lyophilized. After grinding in a Wiley mill, 10 mg of tissue were leached in 5 ml of 10% 5-sulfosalicylic acid for 48 h. Samples were centrifuged at

20,000 X g for 15 min and the supernatant was assayed for NPS according to Rosen (1957). Leucine was used as the standard. NPS values ($\mu\text{M}\cdot\text{g}^{-1}$ dry wt) at 30, 25, 20, and 17.5 ‰ were also divided by the cell volume ratios to yield NPS values expressed as μM NPS per unit of relative cell volume.

Gonads were removed from acclimated adult S. droebachiensis and S. pallidus, fixed in formol-acetic acid-alcohol (Humason, 1972), dehydrated in alcohol and embedded in paraffin. Sections (7 μm) were stained with 1% toluidine blue and examined with a compound microscope.

Coelomic cavity lactic acid levels of 10 acclimated adult S. droebachiensis and S. pallidus from each salinity were measured by the Sigma Diagnostics^R Pyruvate/Lactate assay. Two hundred spawned, nonfertilized eggs of both species at 30, 25, and 20‰ were incubated for 6 h in 0.05, 0.10, 0.15, 0.20 and 0.25 mM lactic acid prior to fertilization. Controls consisted of spawned eggs at each salinity incubated for the same duration in natural, filtered (1.0 μm) seawater. Eggs were rinsed in 2 changes of filtered (1.0 μm) seawater and then fertilized with sperm from males of the same species. Percent fertilization was determined using the criteria above.

METABOLIC RATE STUDIES

Respiration rates of 4 and 8-arm L. variegatus plutei were measured under conditions of acutely declining PO_2 in a closed system respirometer (Strathkelvin^R RC 200 microrespiration

cell equipped with a 1302 oxygen electrode). Sixty-five plutei, in 200 ul of air-saturated seawater, were allowed to acclimate to the apparatus for 1 h. After this time, the respirometer was sealed and the decline in PO_2 was followed on a strip chart recorder connected to a Strathkelvin^R Model 781 Oxygen Meter. Oxygen consumption rates were calculated by converting the overall decline in PO_2 into $ul\ O_2 \cdot g^{-1} \cdot hr^{-1}$, using conversion factors from the nomograph in Strickland and Parsons (1968). Q_{10} values were calculated for respiration rates at each salinity.

One hundred *L. variegatus* plutei were placed into muffled (450°C for 4 hours) chambers containing 100 ul of filtered (0.22 μm) seawater at the appropriate temperature and salinity. Containers filled with seawater only were included as controls. The plutei were allowed to incubate in the seawater for 4-6 hours. Ammonia levels were determined on duplicate samples of incubation water by the phenol-hypochlorite method of Solorzano (1969) as modified by Grasshoff and Johannsen (1972) with ammonium sulfate as the standard. Excretion rates were calculated as: $\mu M\ NH_4 \cdot g^{-1} \cdot hr^{-1}$. Q_{10} values were calculated for excretion rates at each salinity. Oxygen : nitrogen ratios were calculated as: $\mu M\ O_2 \cdot g^{-1} \cdot hr^{-1} / \mu M\ NH_4 \cdot g^{-1} \cdot hr^{-1}$.

STATISTICAL ANALYSIS

The General Linear Model procedure utilizing analysis of variance (SAS Institute Inc., 1985a and 1985b) with Scheffe's values (Steel and Torrie, 1980) as a posteriori hypothesis test

was used in data analysis. One-way analysis of variance comparisons between salinity treatments were made for percent fertilization, developmental rates, survival, cell volume, metabolic rates, NPS and lactic acid levels for all species. Two-way analysis of variance comparisons between temperature and salinity treatments were made for L. variegatus. A probability level of 0.01 was significant for all analyses.

Mean lethal salinities (28 day LC₅₀s) were determined from mortality counts of adults, acclimated embryos, and direct transfer embryos using logit analysis (Silverstone, 1957) when two or more salinities contained both live and dead urchins. The Spearman-Kärber method (Finney, 1971) was used when less than two salinities contained both live and dead urchins.

RESULTS

FERTILIZATION SUCCESS AND CELL VOLUME MEASUREMENTS

The percent fertilization ($\bar{x} \pm \text{S.E.}$) for the direct transfer experiment was $98.3 \pm 2.7\%$ (n=10) for the S. droebachiensis cultures, $97.7 \pm 4.3\%$ (n=10) for the S. pallidus cultures, and $97.5 \pm 4.3\%$ (n=5) for the L. variegatus cultures. The percent fertilization of gametes obtained from acclimated adults significantly decreased in the lower salinities for all species (Tables 1 & 2). All gametes from 3 and 4 wk acclimated S. pallidus were nonviable; therefore, attempts at fertilization

were unsuccessful. S. pallidus were observed spawning after approximately 5-8 days acclimation to 15, 17.5, and 20⁰/ooS. S. droebachiensis gametes acclimated to 17.5, 15 and 10⁰/ooS for 2, 3, and 4 wk were also nonviable. There was also no fertilization of L. variegatus eggs at salinities below 20⁰/oo for any of the acclimation treatments or for the direct transfer cultures. The coelomic fluid of adult urchins at these low salinities was dark red to brown in color, and gave off an offensive odor.

Mean cell volume measurements (Tables 3 & 4; Fig. 1) indicate extensive osmotic swelling of fertilized eggs exposed to low salinity. Extensive swelling is also indicated by the observed decrease in percent volume regulation at the lower salinities for all species (Table 5). Cell volume significantly increased ($P < 0.01$) with decreasing salinity for all species. L. variegatus cells at 15 and 10⁰/ooS lysed before the first cleavage could occur. There was significant swelling of L. variegatus cells at 27.5, 25 and 20⁰/ooS; whereas cells at 30 and 35⁰/ooS did not swell significantly from the controls (Table 3). There was no significant ($P < 0.01$) temperature effect on cell volume. Acclimation to lowered salinity did not reduce cell swelling in S. droebachiensis eggs; however, acclimation did prevent cell lysis at 10⁰/ooS in S. pallidus. S. pallidus embryos exposed to low salinity exhibited more abnormalities in appearance than embryos at 30⁰/oo (Fig. 2).

The fertilization membrane of S. droebachiensis apparently restricts extensive cell swelling. Cells of this species were

observed to swell out to the membrane at 10°/ooS but never lysed. This observation is supported by the percent volume regulation values which decrease down to 2.8-5.8% at 17.5°/ooS and then increase back up to 16.8-36.9% at 10°/ooS (Table 5)

DEVELOPMENTAL RATES AND SURVIVAL TO METAMORPHOSIS

Developmental rates and survival to metamorphosis of all species were significantly affected by salinity (Tables 6 & 7; Figs. 3-5). There was no development at salinities below 20°/oo for any species.

Metamorphosis occurred in cultures of S. droebachiensis at 30, 25, and 20°/ooS for both the direct transfer and acclimation experiments. Only S. pallidus larvae at 30°/ooS survived to metamorphosis (Table 6). Developmental rates and survival to metamorphosis was not significantly affected ($P < 0.01$) by the duration of acclimation at either 20 or 25°/ooS for S. droebachiensis. As previously mentioned, only 2 wk acclimated S. pallidus embryos were obtained; therefore, only the 2 wk acclimation data for both species are tabulated (Table 6). There was no significant difference in developmental rates between direct transfer cultures and acclimated cultures (Table 6); however, larval survival to metamorphosis of both S. droebachiensis and S. pallidus was significantly lower in the the 2 wk acclimated cultures than in the direct transfer cultures (Figs. 3 & 4).

There was no significant difference in the calculated

28 day LC₅₀ values of direct transfer embryos and acclimated embryos of S. pallidus; however, both values for embryos were significantly higher ($P < 0.01$) than the corresponding value for adults (Fig. 4). The LC₅₀ value for acclimated S. droebachiensis embryos was significantly higher than the value for direct transfer embryos indicating that for this species exposure of embryos to low salinity for an extended period was detrimental (Fig. 3). The LC₅₀ value for acclimated adult S. droebachiensis was significantly lower than the corresponding values for both embryo cultures.

The development of L. variegatus is affected by both temperature and salinity (Table 7; Fig. 5); however, the calculated Q₁₀ values and the Scheffe's values indicate that temperature is more influential on developmental rates than the 5‰ difference in salinity. The Q₁₀ values for days to metamorphosis at 30 and 35‰ were 0.380 and 0.384 respectively. The developmental rates of L. variegatus at 30 and 35‰ at both temperatures were very synchronous (Fig. 6). The synchrony of development was significantly decreased at 27.5‰ (Fig. 6). The developmental rates varied directly with salinity and temperature for all cultures. There was no development at salinities less than 27.5‰ (Table 7) at either temperature. At 27.5‰, development ceased at the 4-arm pluteus stage and all larvae died within 4-5 days after this stage was reached. Settlement and metamorphosis (Fig. 7) of L. variegatus occurred only in plutei at 30 and 35‰ at both temperatures (Table 7).

There was a significantly higher (ANOVA; $P < 0.01$) survival to metamorphosis at 35 than at $30^{\circ}/\text{ooS}$ at both temperatures. There was no significant temperature effect on survival of plutei to metamorphosis. There was a significantly higher (ANOVA; $P < 0.01$) percentage of abnormal L. variegatus plutei at $30^{\circ}/\text{oo}$ ($12.3 \pm 0.5\%$) than at $35^{\circ}/\text{ooS}$ ($9.2 \pm 0.8\%$). Almost all of the plutei at $27.5^{\circ}/\text{ooS}$ ($98.7 \pm 1.5\%$) were very abnormal in appearance when compared to larvae at the two higher salinities (Fig. 8).

The 28 day LC_{50} for adult L. variegatus was determined to be $18^{\circ}/\text{ooS}$ (Fig. 5). Acclimation of adult urchins to 1, 2 or 4 weeks at lowered salinity did not enhance larval survival or alter the developmental patterns of this species from that observed with the direct transfer experiment at the same culture salinity (Fig. 5) (ANOVA; $P < 0.01$). Larval developmental rates and survival data were so similar that they were virtually indistinguishable from the direct transfer data.

The juvenile L. variegatus which were back-transferred to the various salinity regimes at 23°C , exhibited no enhanced survival (Table 8) over adult urchins exposed to the same salinities (Fig. 5). By one week after transfer there were no urchins alive at $15^{\circ}/\text{oo}$ or lower salinities. The percent survival of juvenile urchins at the higher salinities had not changed significantly (ANOVA; $P < 0.01$) by one week from the values calculated at four days (Table 8).

HISTOLOGY, NPS, AND LACTATE ANALYSIS

Histological examination revealed no observable differences in gonadal structure between adult urchins acclimated to high and low salinity (Fig. 9).

There was no significant effect of acclimation duration on ninhydrin-positive substances or lactic acid levels for either S. droebachiensis or S. pallidus (Tables 9 & 10; Fig. 10). NPS values of the gonads from acclimated adults of both species were significantly lower at 17.5‰ than corresponding values at the higher salinities (Table 9; Fig. 10). There was no significant difference in gonadal NPS values at 25 and 30‰ (Table 9).

Coelomic cavity lactic acid levels varied indirectly with salinity for adult S. droebachiensis and S. pallidus (Table 10). Lactic acid levels of urchins at 20‰ were significantly higher than corresponding levels at 25 and 30‰ for both species.

The exposure of unfertilized eggs of S. droebachiensis and S. pallidus to increasing concentrations of lactic acid resulted in a significant decrease in percent fertilization (Fig. 11). The percent fertilization of eggs of S. droebachiensis at 20‰ exposed to 0.15 mM lactate was 66% of the control value at the same salinity. This concentration of lactic acid was 0.025 mM below that found in the coelomic fluid of adult urchins after 2 wk acclimation (Table 10). Similarly, the percent fertilization of the eggs of S. pallidus at 20‰ and 0.15 mM lactate was 40% of the control value at the same salinity.

METABOLIC RATES

In the declining oxygen tension experiment, L. variegatus plutei extracted all detectable oxygen from the respirometer in all temperature - salinity conditions. There was a significant temperature effect on respiration rates at both 30 and 35°/ooS for both 4 and 8-arm plutei (Fig. 12; Table 11). Temperature did not significantly affect the respiration rates at 27.5°/ooS for the 4-arm plutei. Respiration rates were significantly depressed at 27.5°/ooS for the 4-arm plutei. Respiration rates were not significantly different between 30 and 35°/ooS for the 4-arm plutei; however, the rates were different between the two salinities for the 8-arm plutei.

Ammonia excretion rates of L. variegatus plutei were significantly different at both temperatures for all salinities (Fig. 12; Table 11). The excretion rates were significantly higher at 27.5 than at 30 or 35°/ooS for both temperatures.

L. variegatus plutei at 27.5°/ooS possessed a significantly lower O:N ratio than larvae at the higher salinities for both temperatures (Fig. 12). There was no significant difference in O:N ratios between 30 and 35°/ooS at either temperature for 4-arm or 8-arm plutei (Fig. 12) with mean values ranging from 12.5 to 4.7 indicating an increased reliance on protein catabolism at 27.5°/ooS.

DISCUSSION

The zone of lethality (zone of resistance adaptation) (Vernberg and Vernberg, 1972; Prosser, 1986) for salinity of larval L. variegatus, S. droebachiensis and S. pallidus is well within the observed tolerance limits of the adults as suggested by the present data and by previous studies (Moore et al., 1963; Sabourin and Stickle, 1981; Klinger et al., 1986; Stickle and Diehl, 1987). The zone of lethality is the extreme of an environmental factor gradient (i.e., salinity) which results in the organism's death and can be used as a measure of the resistance adaptation of various populations. The larval stages of all three species are the most sensitive life history stage with respect to salinity gradients, as is true for many groups of invertebrates including molluscs (Calabrese and Davis, 1970; Watts et al., 1982; Roller and Stickle, 1985, 1987). Since acclimation of sea urchins from high salinity habitats to low salinity does not enhance larval survival, our understanding of the mechanisms responsible for populations adapted to low and variable salinity must await further studies on these organisms.

Apparently, the rate of transfer of echinoderms to reduced salinity has a minimal effect on their salinity tolerance (Stickle and Diehl, 1987). Directly transferred Asterias forbesi individuals had the same salinity tolerance as individuals in which the salinity was gradually reduced (Loosanoff, 1945). The same 14 day LC₅₀ of adult S. droebachiensis is obtained whether individuals are directly transferred or stepwise adapted to the

final salinity (Sabourin and Stickle, 1981).

Roller and Stickle (1985) demonstrated the relationship between the early period of larval tolerances to salinity and adult distribution along salinity gradients for four species of echinoderms from the Friday Harbor area. The present data on salinity tolerance of larval S. droebachiensis and S. pallidus to metamorphosis also correlates well with the distributional pattern of the adult stages. Strongylocentrotus droebachiensis is a circumpolar sea urchin which tolerates environmental salinities as low as 13‰ from both the low and high salinity environments of Juneau, Alaska and Friday Harbor, respectively (Sabourin and Stickle, 1981; Stickle and Diehl, 1987). The developmental rates and larval tolerances to metamorphosis indicate that while the embryonic stages of S. droebachiensis are more stenohaline and fall well within the tolerance limits of the adults, the larvae are able to tolerate a relatively wide range of salinity gradients with over 50 to 90% surviving to metamorphosis at 20 and 30‰ respectively. S. pallidus are usually restricted to depths greater than 50 m in the San Juan Archipelago (Kozloff, 1974), where the ambient salinity is always greater than 30‰ (Thomson, 1981; Collias et al., 1974). The larvae of this species are very stenohaline and will not survive salinities less than 27.5‰ (Roller and Stickle, 1985).

Lytechinus variegatus is an abundant urchin along the southeastern coast of the United States. L. variegatus ranges from North Carolina to Brazil on the American coast and is also

found off Bermuda and the Cape Verde Islands (Moore et al., 1963). L. variegatus is normally found in seawater ranging from one foot in depth to greater than 30 feet (J. Lynn, personal communication). The normal salinity in the area where adults were collected varies from 25 to 35‰, whereas, the temperature normally varies from 17 to 30°C (J. Lynn, personal communication; Moore et al., 1963).

The pelagic period of larval L. variegatus is typical of temperate species (Thorson, 1950); however, this suggests that the dispersal of larvae of this species is probably not as extensive as for larvae from more northern latitudes (Thorson, 1950; Strathmann, 1978). Species such as S. droebachiensis and S. pallidus, which possess a long pelagic larval period, would be more vulnerable to any variations in environmental conditions than species such as L. variegatus, which spend less time in the water column (Thorson, 1950; Strathmann, 1978).

Results from a previous investigation suggest that salinity tolerance of the early stages of echinoderm larvae may be modified by acclimating adults to reduced salinities and that the ova are more strongly influenced by low salinity than spermatozoa. Gezelius (1963) found that the cleavage rate of fertilized Psammechinus miliaris eggs at a given temperature was related to the salinity at which the parents had been adapted. The present data indicate that the acclimation of adult S. droebachiensis, S. pallidus, and L. variegatus to lowered salinity does not enhance the subsequent development or survival

of embryonic stages to metamorphosis. In this study larval mortality was frequently preceded by the appearance of abnormal larvae in later development as shown by death of L. variegatus at the 4-arm pluteus stage 10-13 days after fertilization at 27.5°/ooS. Delayed mortality of this nature would not be evident when studying only the cleavage rate of fertilized eggs (Gezelius, 1963).

Since echinoderms possess a permeable body wall (Binyon, 1966, 1972) and are osmoconformers, the gonads in the coelomic cavity are not protected from osmotic changes and are therefore exposed to salinity changes in the environment. This condition is unlike that found in osmoregulating marine fish which may offer osmotic protection to gametes (Hoar, 1969); therefore, the preincubation of gametes to lowered salinity by the acclimation of adult urchins may only offer the advantage of time for gametes to become isosmotic to reduced salinity. However, volume regulation of fertilized ova was minimal as shown by only 22% regulation for S. droebachiensis, 2% for S. pallidus, and 0% for L. variegatus at 2 weeks acclimation to 20°/ooS (Table 5).

In most marine invertebrates, the isosmolarity of the body fluids with seawater is established by inorganic ions; whereas, within cells osmotic balance is maintained partly by inorganic ions and small-molecular weight organic compounds (Lange, 1964). High intracellular concentrations of amino acids have been demonstrated in many marine invertebrates (Lange, 1964; Baginski and Pierce, 1978; Burton and Feldman, 1982; Kapper et

al., 1985; Stickle et al., 1985; Stickle and Diehl, 1987). When an animal is transferred from a high salinity to a lower salinity environment, and thus exposed to hyposmotic shock, cell volume is initially increased by the osmotic influx of water. This phase is followed by a reduction in intracellular concentrations of some amino acids in response to the reduced external osmolarity (Rankin and Davenport, 1981; Diehl, 1986). Lange (1964) found that the total intestinal ninhydrin-positive substances of adult S. droebachiensis correlated linearly with the seawater salinity. The NPS levels of the ovaries of adult urchins in the present study showed a significant decrease upon 2, 3, and 4 wk acclimation to salinities below 25-30‰, thus indicating the possible expulsion of intracellular amino acids in response to hyposmotic stress. The relationship between the seawater salinity and total NPS on a relative cell volume basis is largely passive due to water logging at low salinity as observed in the present investigation and by Lange (1964). There was, however, no obvious evidence of tissue damage from histological data, most likely due to more subtle physiological effects.

Bookbinder and Shick (1986) found that S. droebachiensis ovaries have a high capacity for the production of large amounts of lactate under imposed anoxia; however, they also found that lactate accounted for only 37% of the total anoxic heat dissipation, which suggested that other end products may be present. The increase in coelomic cavity lactic acid levels of

adult urchins upon exposure to low salinity observed in the present investigation may be indicative of a more extensive anaerobic environment for the entire gonad, not just its interior as a result of perfusion limitations, which in turn may be detrimental to the viability of gametes. These conditions resulted in a reduction of the viability of S. droebachiensis and S. pallidus gametes in individuals exposed to reduced salinity and preincubated in lactic acid solutions similar to concentrations found in sea urchins.

Previous studies have provided a theoretical framework for investigations of the responses of marine invertebrates to variations in environmental factors (Fry, 1947, 1971; Precht, 1958; Kinne, 1964; Prosser, 1986). Variations in environmental factors can result in physiological stress for many organisms (Bayne et al., 1979; Widdows et al., 1981; Roller and Stickle, 1985; Stickle, 1985; Roller and Stickle, 1987; Stickle and Diehl, 1987). Bayne (1985) defined stress as "an environmental stimulus, which by exceeding a threshold value, disturbs normal animal function."

The developmental period of larvae prior to metamorphosis to a benthic existence is an extremely important attribute of larval "wastage" as described by Thorson (1950). According to Thorson (1950), the mortality or waste of larvae is controlled by their dependance on the plankton as a source of food and by the time they spend in the plankton. Species such as S. droebachiensis and S. pallidus, which have a prolonged planktonic

existence 50-151 days (Strathmann, 1978), would have a greater larval wastage than a species such as Spirorbis borealis, which spend only a few hours in the plankton (Thorson, 1950). The longer a larva is in the water column, the greater is the possibility of encountering predators or detrimental environmental conditions, such as salinity fluctuations. In addition, the results from the present study indicate that low salinity prolongs the length of the planktonic existence of echinoderm larvae, thus increasing possible larval wastage. Based on this observation, and since L. variegatus has a shorter pelagic existence, one might speculate that L. variegatus might have less total larval wastage than S. droebachiensis even though S. droebachiensis plutei are more euryhaline.

Oxygen : nitrogen ratios have been used as a general index of stress in various marine organisms (Bayne, 1985; Bayne et al., 1979; Widdows et al., 1981, Shirley and Stickle, 1982; Stickle, 1985). The lower respiration rates observed in the present investigation, coupled with the enhanced excretion rates, resulted in depressed O:N ratios of 4 and 8-arm plutei at 27.5°/ooS which in turn indicates an increased reliance on protein as a metabolic substrate. Physiological stress induces protein catabolism with a resulting decrease in O:N ratio. Increased reliance on protein catabolism is likely due to reduced feeding at 27.5°/ooS, because the respiration rate is reduced.

It is accepted that ectothermic metabolism is complicated with various temperature ranges associated with different

systemic states often involving specific ionic arrangements and alternative enzymatic patterns (Wieser, 1973). Many organisms exhibit a typical temperature relationship with a Q_{10} of approximately 2 over the entire biological range (Valen, 1958; Fuhrman and Fuhrman, 1959; Mangum and Sassaman, 1969). A rise of 10°C in temperature generally results in a two or three-fold increase in metabolic rate (Schmidt-Nielsen, 1980). The Q_{10} values obtained in the present study are indicative of increased metabolic rates at higher temperatures. The Q_{10} values, in conjunction with the developmental rates, metabolic rates, and survival data, indicate that for L. variegatus, salinity appears to be the main factor in determining larval survival, thus influencing the distribution of the species. Temperature, however, seems to be the controlling factor of developmental rates during early embryogenesis.

Previous attempts to culture L. variegatus embryos at $27-28^{\circ}\text{C}$ resulted in cultures which exhibited high mortality during early embryogenesis. Furthermore, none survived to settlement and metamorphosis. Our data indicate that the best temperature range for culturing L. variegatus in terms of optimal developmental rates and survival to metamorphosis appears to be $23-25^{\circ}\text{C}$.

The present data, in conjunction with a previous study (Roller and Stickle, 1985), indicate that both adult and larval S. pallidus and L. variegatus are more stenohaline than corresponding S. droebachiensis life stages; however, the larval

stages of all three species are distinctly more sensitive to low salinity variations than are adults. Temperature was more important in varying developmental rates of L. variegatus; whereas, salinity was more influential on overall survival to metamorphosis. Osmotic shock, as determined from the cell volume data, suggest that the greatest mortality due to salinity stress occurs during early embryogenesis at salinity extremes, but abnormal development occurs later in larval life at slightly higher salinities. In addition, data obtained from the back-transfer of juveniles correlates well with the tolerance limits of adults. Approximately 84% of the back-transferred juveniles survived at 20°/ooS, whereas none survived at the lower salinities. The LC₅₀ data indicate that 18°/ooS is the lower tolerance limit for L. variegatus. The larval tolerance limits of the three species correlates well with the known distribution of adults. There was no advantage to larval survival in low salinities incurred by acclimating adult urchins to hyposmotic conditions. These data suggest that the zone of lethality for larval urchins may possibly limit the distribution of adults along salinity gradients.

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Table 1. % Fertilization of Eggs from Acclimated Adult Urchins

Species	Acclimation		Salinity (‰)			
	Time	25	20	17.5	15	10
<u>S. droebachiensis</u>	2wk	90.7 ± 5.2 (A)*	82.9 ± 2.8 (BC)	0	0	0
	3wk	92.2 ± 3.8 (A)	81.3 ± 4.2 (CD)	0	0	0
	4wk	85.6 ± 4.8 (BC)	76.7 ± 5.3 (D)	0	0	0
<u>S. pallidus</u>	2wk	71.2 ± 3.9 (A)	41.4 ± 5.4 (B)	0	0	0
	3wk	0	0	0	0	0
	4wk	0	0	0	0	0

* - Scheffe Values for comparisons between salinities. Values with the same letter are not significantly different ($P < 0.01$)

TABLE 2.% FERTILIZATION OF EGGS FROM ACCLIMATED ADULT L. VARIEGATUS

ACCLIMATION TIME	SALINITY (‰)				
	35	30	27.5	25	20
1 Week	97.9 ± 4.6 (A)*	89.3 ± 4.7(B)	54.7 ± 5.7(C)	10.2 ± 2.4(D)	11.3 ± 5.6 (D)
2 Weeks	95.2 ± 4.9 (A)	90.2 ± 2.3(B)	57.3 ± 4.2 (C)	11.3 ± 3.7(D)	10.8 ± 3.8(D)
3 Weeks	98.2 ± 5.7 (A)	88.6 ± 4.7(B)	61.4 ± 3.9(C)	9.8 ± 4.4 (D)	8.9 ± 4.9(D)

*Scheffe Values for comparisons between salinities.

Means with the same letter are not significantly different (P<0.01).

TABLE 3. MEAN CELL VOLUME ($\mu\text{l} \times 10^{-3}$) ($\bar{x} \pm \text{SE}; n=20$) OF LYTECHINUS VARIEGATUS*

ACCLIMATION TIME	ACCLIMATION SALINITY (‰)	TRANSFER SALINITY (‰)				
		35	30	27.5	25	20
1 wk	35	0.820 \pm 0.051(D)	0.846 \pm 0.037(D)	0.966 \pm 0.033(C)	1.253 \pm 0.053(B)	1.371 \pm 0.025(A)
	30	0.801 \pm 0.068(D)	0.851 \pm 0.036(D)	0.951 \pm 0.021(C)	1.194 \pm 0.042(B)	1.383 \pm 0.043(A)
	27.5	0.862 \pm 0.033(D)	0.842 \pm 0.042(D)	0.968 \pm 0.023(C)	1.201 \pm 0.063(B)	1.377 \pm 0.011(A)
	25	0.860 \pm 0.041(D)	0.811 \pm 0.069(D)	0.933 \pm 0.013(C)	1.182 \pm 0.021(B)	1.363 \pm 0.024(A)
	20	0.886 \pm 0.021(D)	0.824 \pm 0.057(D)	0.954 \pm 0.024(C)	1.263 \pm 0.064(B)	1.448 \pm 0.035(A)
2 wk	35	0.861 \pm 0.032(D)	0.880 \pm 0.021(D)	0.951 \pm 0.022(C)	1.234 \pm 0.072(B)	1.382 \pm 0.021(A)
	30	0.801 \pm 0.061(D)	0.866 \pm 0.034(D)	0.929 \pm 0.011(C)	1.248 \pm 0.043(B)	1.374 \pm 0.024(A)
	27.5	0.840 \pm 0.046(D)	0.880 \pm 0.023(D)	0.932 \pm 0.014(C)	1.266 \pm 0.042(B)	1.357 \pm 0.028(A)
	25	0.802 \pm 0.071(D)	0.823 \pm 0.057(D)	0.931 \pm 0.015(C)	1.210 \pm 0.063(B)	1.358 \pm 0.028(A)
	20	0.811 \pm 0.062(D)	0.820 \pm 0.043(D)	0.978 \pm 0.033(C)	1.194 \pm 0.077(B)	1.364 \pm 0.024(A)
4 wk	35	0.796 \pm 0.094(D)	0.852 \pm 0.041(D)	0.943 \pm 0.042(C)	1.191 \pm 0.063(B)	1.374 \pm 0.026(A)
	30	0.827 \pm 0.073(D)	0.864 \pm 0.036(D)	0.957 \pm 0.036(C)	1.262 \pm 0.047(B)	1.388 \pm 0.021(A)
	27.5	0.799 \pm 0.098(D)	0.827 \pm 0.063(D)	0.964 \pm 0.053(C)	1.273 \pm 0.061(B)	1.373 \pm 0.024(A)
	25	0.843 \pm 0.041(D)	0.866 \pm 0.047(D)	0.935 \pm 0.011(C)	1.187 \pm 0.055(B)	1.367 \pm 0.036(A)
	20	0.827 \pm 0.055(D)	0.845 \pm 0.042(D)	0.943 \pm 0.053(C)	1.221 \pm 0.043(B)	1.394 \pm 0.042(A)

* - All cells at 15 & 10 ‰ S lysed.

TABLE 4. CELL VOLUME ($\mu\text{l} \times 10^{-3}$) ($\bar{x} \pm \text{SE}; n=10$) OF S. DROEBACHIENSIS AND S. PALLIDUS ACCLIMATED TO 5 SALINITIES FOR 2, 3, & 4 WEEKS.

SPECIES	ACCLIMATION SALINITY (‰)	ACCLIMATION TIME (WEEKS)		
		2	3	4
<u>S. droebachiensis</u>	30	2.56 \pm 0.12(D)*	2.78 \pm 0.21(D)	2.47 \pm 0.34(D)
	25	2.76 \pm 0.21(D)	2.89 \pm 0.20(D)	2.59 \pm 0.15(D)
	20	3.56 \pm 0.32(C)	3.44 \pm 0.41(C)	3.29 \pm 0.27(C)
	17.5	4.32 \pm 0.21(B)	4.64 \pm 0.16(B)	4.19 \pm 0.25(B)
	10	6.43 \pm 0.34(A)	6.29 \pm 0.36(A)	6.58 \pm 0.29(A)
<u>S. pallidus</u>	30	2.52 \pm 0.36(C)	2.39 \pm 0.42(C)	2.43 \pm 0.40(C)
	25	2.81 \pm 0.29(C)	2.77 \pm 0.39(C)	2.62 \pm 0.20(C)
	20	3.76 \pm 0.21(B)	3.59 \pm 0.15(B)	3.72 \pm 0.17(B)
	17.5	4.30 \pm 0.21(A)	4.01 \pm 0.18(A)	4.28 \pm 0.12(A)
	10	-----**	-----	-----

*Scheffe values calculated across salinities by week for each species. Means with the same letter are not significantly different ($P < 0.01$).

**Cells lysed

TABLE 5. PERCENT VOLUME REGULATION FOR FERTILIZED EGGS OF S. DROEBACHIENSIS, S. PALLIDUS, AND L. VARIEGATUS.

SPECIES	ACCLIMATION TIME	SALINITY (‰)						
		10	17.5	20	25	27.5	30	35
<u>S. droebachiensis</u>	2 wk	24.5	2.8	22	60	---	*	---
	3 wk	36.9	5.8	52	80	---	*	---
	4 wk	16.8	2.8	34	76	---	*	---
<u>S. pallidus</u>	2 wk	**	0	2	40	---	*	---
	3 wk	**	4.5	0	20	---	*	---
	4 wk	**	0	0	60	---	*	---
<u>L. variegatus</u>	1 wk	---	---	0	0	34.1	77.2	*
	2 wk	---	---	0	0	70	96.4	*
	4 wk	---	---	0	0	22.7	49.1	*

* - Reference Salinity - No Data

** - No Data - Cells Lysed

--- - No Data

TABLE 6. Elapsed Days to Developmental Stage

STAGE	<i>S. droebachiensis</i>						<i>S. pallidus</i>					
	30‰S		25‰S		20‰S		30‰S		25‰S		20‰S	
	Direct Transfer	2 wk Acclimation	Direct Transfer	2 wk Acclimation	Direct Transfer	2 wk Acclimation	Direct Transfer	2 wk Acclimation	Direct Transfer	2 wk Acclimation	Direct Transfer	2 wk Acclimation
2 Cell	0.12	0.17	0.12	0.17	0.12	0.21	0.10	0.12	0.10	0.12	0.12	0.14
8-16 Cell	0.27	0.33	0.29	0.33	0.29	0.33	0.25	0.29	0.25	0.38	0.41	0.45
Hatched Blastula	0.75	0.86	0.75	0.86	1.0	1.0	0.75	0.82	1.2	1.3	1.5	1.5
Prism	3	3.5	3.5	3.5	4	4	3	3.5	4	†	†	†
4-Arm Pluteus	5	6	6	6	7	7.5	6	6	†	†	†	†
6-Arm Pluteus	12	12.5	13	13.5	14	14	13	14	†	†	†	†
8-Arm Pluteus	17	17	19	20	21	22	19	20	†	†	†	†
Echinus Rudiment	35	37	42	45	48	50	40	46	†	†	†	†
Metamorphosis	58(C)*	59(C)	64(B)	63(B)	74(A)	79(A)	66(B)	69(B)	†	†	†	†

† - no data, dead culture

* - Scheffe Values for comparisons between salinities. Values with the same letter are not significantly different ($P < 0.01$)

TABLE 7. DAYS TO DEVELOPMENTAL STAGE OF L. VARIEGATUS

STAGE	23°C			18°C		
	SALINITY(‰)			SALINITY(‰)		
	35	30	27.5	35	30	27.5
Fertilized egg	0	0	0	0	0	0
1st cleavage	0.06	0.17	0.17	0.54	0.62	0.98
8-16 Cell	0.18	0.42	0.79	1	1	1.5
Hatched blastula	0.17-1.0	2-3	4-6	1.5-2	2-2.5	2-2.5
Late gastrula	1.5-2.0	3-4	6-7	2-3	5-6	6-7
4-Arm Pluteus*	4-5	5-6	10-11	7-8	11-12	12-13
4-Arm Pluteus**	5-6	6-8	—†	9-11	13-15	—†
6-Arm Pluteus	8-12	13-17	—	14-15	17-18	—
8-Arm Pluteus	15-17	20-23	—	21-25	22-25	—
Echinus rudiment(w/primary podia)	20-21	25-26	—	29-31	33-35	—
Metamorphosis	24-25 (A)	27-31 (A)	—	37-42 (B)	45-49 (B)	—

* Total Length = 250μm

** Total Length = 500μm

† No data - Dead Culture

() Values with the same letter are not significantly different (P<0.01)

TABLE 8. % SURVIVAL OF JUVENILE L. VARIEGATUS 4 DAYS AFTER BACK-TRANSFER TO LOWER SALINITIES†

ORIGINAL CULTURE SALINITY (‰)	TRANSFER SALINITIES (‰)						
	35	30	27.5	25	20	15	
30	96.1 ± 2.7 (A)	95.2 ± 1.3 (A)*	96.8 ± 3.2 (A)	90.6 ± 4.3 (B)	84.7 ± 1.1 (C)	1.5 ± 0.3 (D)	0
35	95.6 ± 1.4 (A)*	95.9 ± 4.3 (A)	94.8 ± 4.2 (A)	89.9 ± 3.3 (B)	83.8 ± 1.2 (C)	2.7 ± 0.7 (D)	0

† $\bar{x} \pm SE$, N=200 Urchins/Treatment

* Controls

() Scheffe values - Means with the same letter are not significantly different ($P < 0.01$)

TABLE 9. NPS ($\mu\text{M}\cdot\text{g}^{-1}$ Dry wt) VALUES FOR OVARIES OF S. DROEBACHIENSIS AND S. PALLIDUS AFTER 2,3, & 4 WEEKS ACCLIMATION TO 17.5, 20, 25, & 30 ‰ S*

SPECIES	ACCLIMATION SALINITY	ACCLIMATION TIME (WEEK)		
		2	3	4
<u>S. droebachiensis</u>	30	263.8 \pm 14.8(A)	274.6 \pm 26.7(AB)	299.5 \pm 40.2(A)
	25	242.3 \pm 21.6(A)	317.7 \pm 29.3(A)	325.1 \pm 32.3(A)
	20	217.8 \pm 14.7(B)	269.4 \pm 10.4(B)	261.0 \pm 11.2(B)
	17.5	206.3 \pm 10.8(B)	228.6 \pm 14.3(C)	214.3 \pm 21.1(C)
<u>S. pallidus</u>	30	487.6 \pm 21.6(A)	517.4 \pm 45.8(A)	526.3 \pm 25.2(B)
	25	421.7 \pm 20.3(B)	569.8 \pm 35.7(A)	563.4 \pm 48.3(AB)
	20	432.8 \pm 14.8(B)	594.3 \pm 48.7(A)	586.2 \pm 19.5(A)
	17.5	414.3 \pm 11.7(B)	448.6 \pm 21.8(C)	347.3 \pm 42.4(C)

*Scheffe values calculated across salinities by week for each species.

Means with the same letter are not significantly different ($P < 0.01$).

TABLE 10. COELOMIC LACTATE LEVELS (mM/l) ($\bar{x} \pm SE$; n=10) OF ADULT S. DROEBACHIENSIS AND S. PALLIDUS ACCLIMATED TO 30, 25, & 20‰ S FOR 2, 3, & 4 WEEKS.

SPECIES	ACCLIMATION TIME	SALINITY (‰)		
		30	25	20
<u>S. droebachiensis</u>	2 wk	0.0801 \pm 0.0074(H)*	0.0903 \pm 0.0088(FGH)	0.1392 \pm 0.0088(D)
	3 wk	0.0906 \pm 0.0092(FGH)	0.0942 \pm 0.0073(FGH)	0.1694 \pm 0.0083(C)
	4 wk	0.0823 \pm 0.0098(GH)	0.1004 \pm 0.0090(F)	0.1866 \pm 0.0078(B)
<u>S. pallidus</u>	2 wk	0.0834 \pm 0.0079(GH)	0.0994 \pm 0.0082(FG)	0.1772 \pm 0.0074(BC)
	3 wk	0.0797 \pm 0.0088(H)	0.1192 \pm 0.0073(E)	0.2073 \pm 0.0087(A)
	4 wk	0.0861 \pm 0.0062(GH)	0.1255 \pm 0.0096(DE)	0.1991 \pm 0.0098(AB)

*Scheffe values calculated for each salinity. Means with the same letter are not significantly different.

TABLE 11. Q_{10} VALUES FOR METAMORPHOSIS TIMES, RESPIRATION RATES,
AMMONIA EXCRETION RATES FOR L. VARIEGATUS PLUTEI

	4-ARM PLUTEI			8-ARM PLUTEI	
	SALINITY(‰)			SALINITY(‰)	
	27.5	30	35	30	35
Respiration Rates	1.46	2.07	2.32	2.25	2.90
Excretion Rates	2.85	2.25	2.90	2.54	1.94

Figure 1. Cell volume measurements for eggs of S. droebachiensis and S. pallidus 4 hours after fertilization. Means with the same letter are not significantly different ($P < 0.01$; $n = 30$ eggs per treatment).

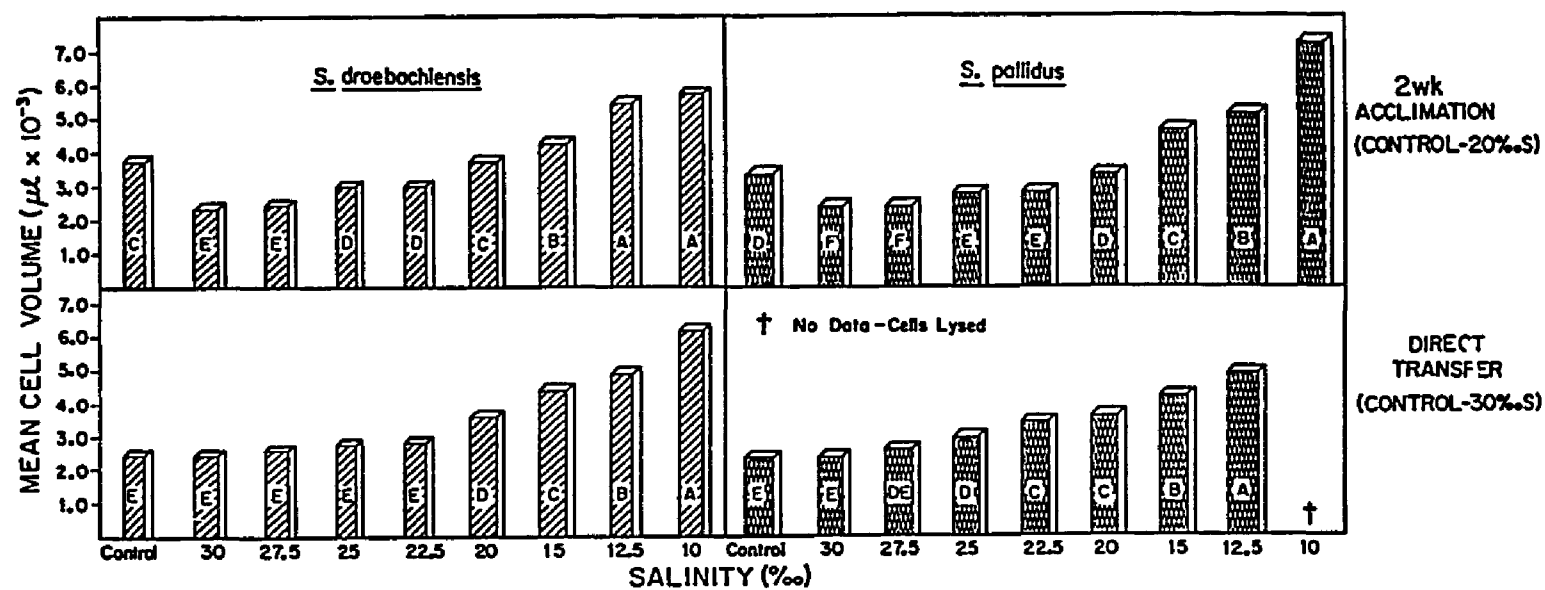


Figure 2. Normal blastula (a) and abnormal embryo (b) of S. pallidus illustrating salinity effects on development. The normal embryo was cultured at 30°/ooS. The abnormal embryo was obtained from adults which had been acclimated to 20°/ooS for 2 wk.

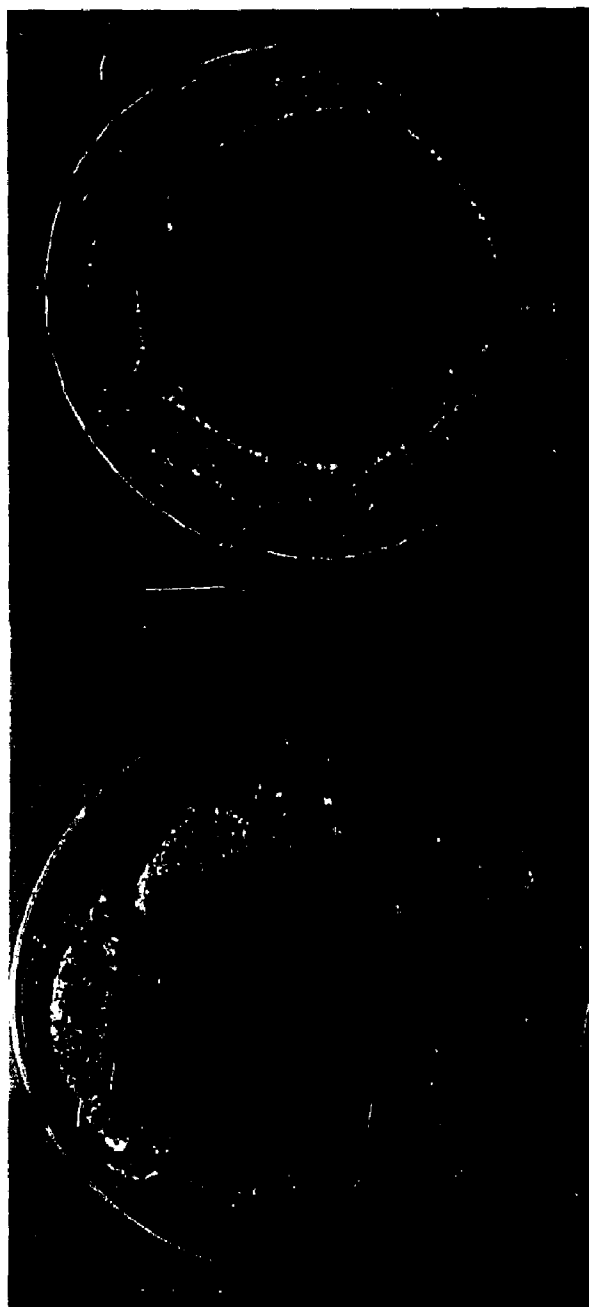


Figure 3. Twenty eight day LC₅₀ values (°/oo) for direct transfer embryos, acclimated embryos and acclimated adults of S. droebachiensis. 95% confidence intervals were smaller than symbols used. Means with the same letter are not significantly different (P<0.01).

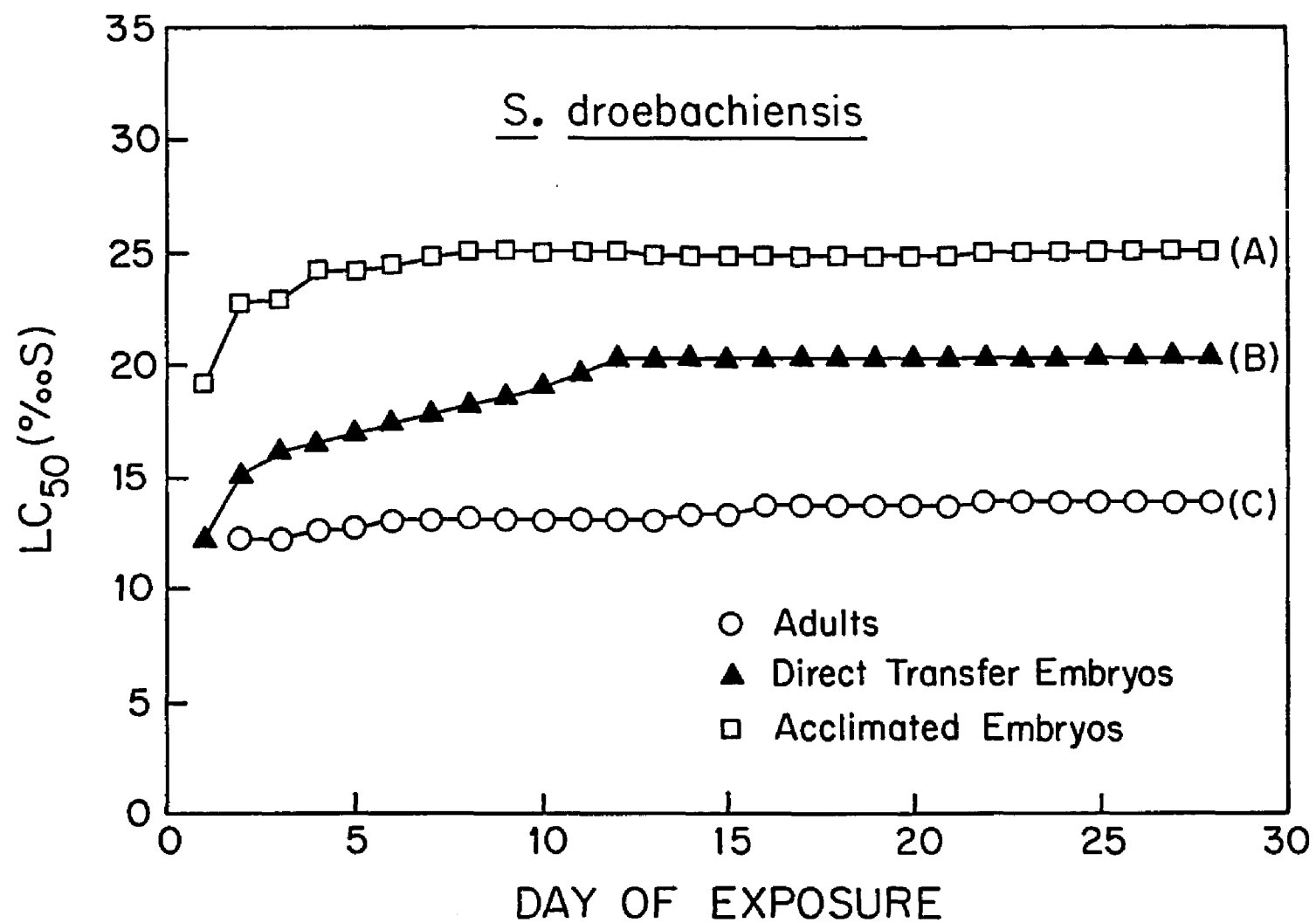


Figure 4. Twenty eight day LC₅₀ values (°/oo) for direct transfer embryos, acclimated embryos and acclimated adults of S. pallidus. 95% confidence intervals were smaller than symbols used. Means with the same letter are not significantly different (P<0.01).

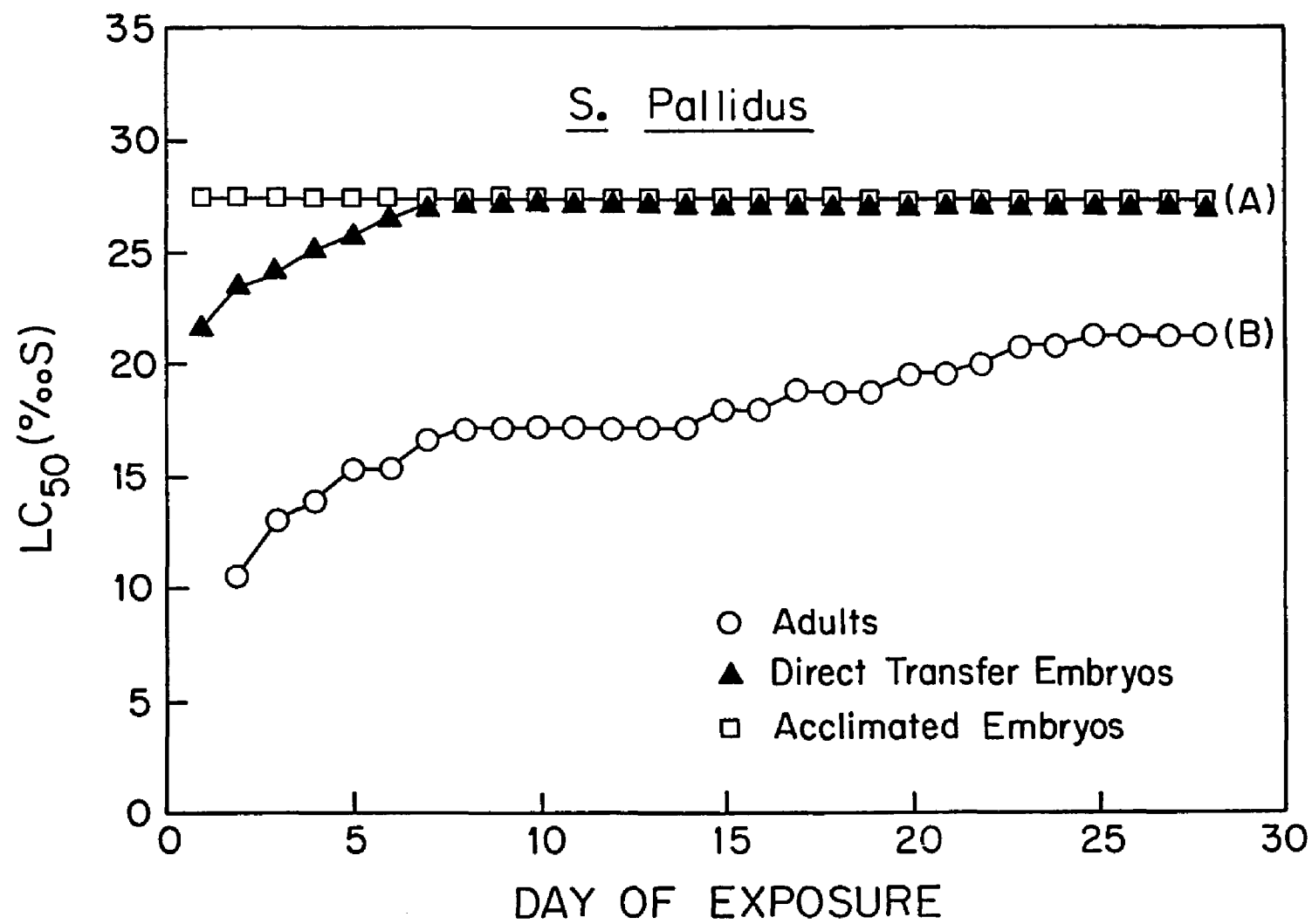


Figure 5. Twenty eight day LC₅₀ values (°/oo) for direct transfer embryos, acclimated embryos and acclimated adults of L. variegatus. 95% confidence intervals were smaller than symbols used. Means with the same letter are not significantly different (P<0.01).

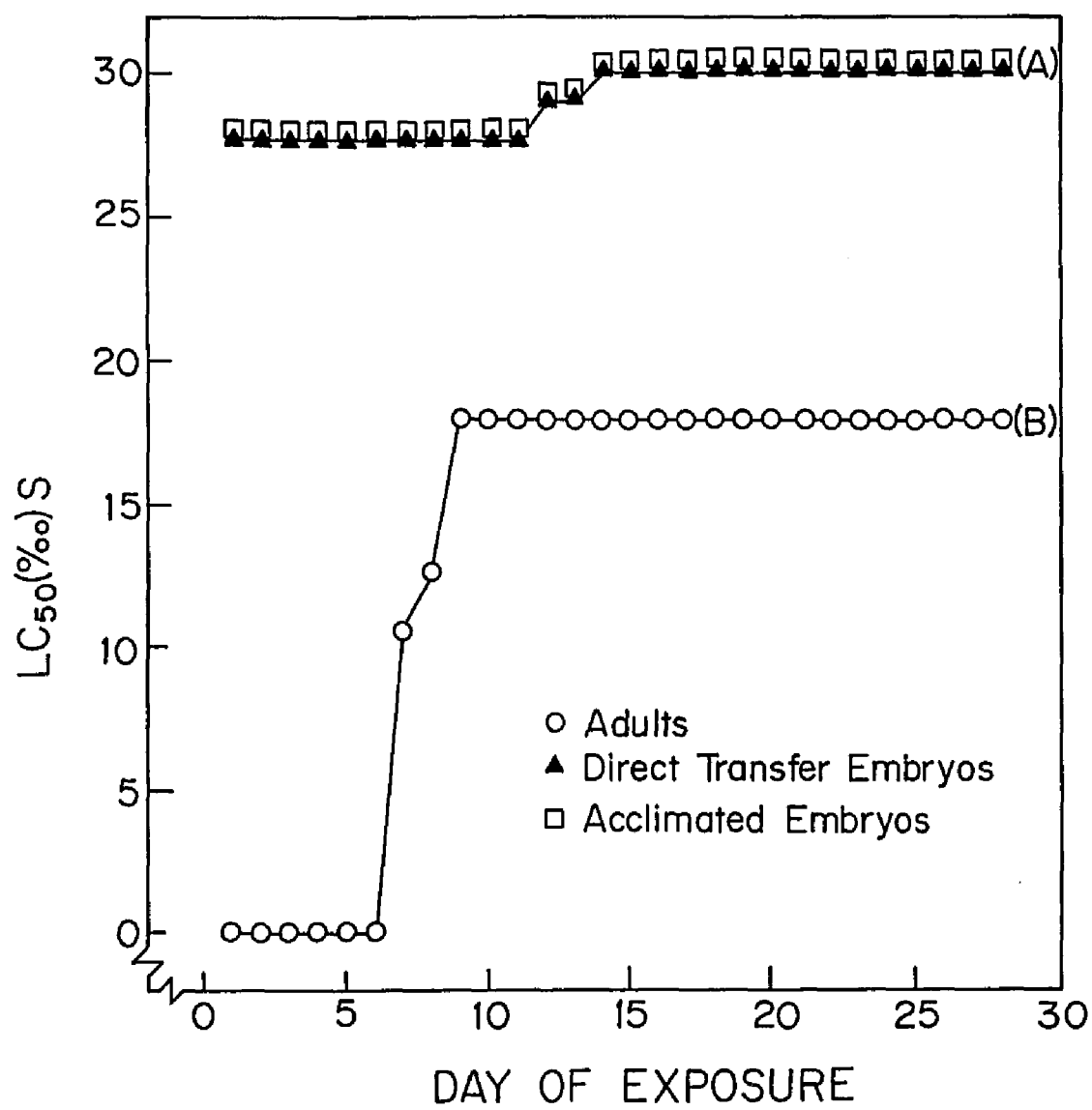


Figure 6. Percent synchrony of development of *L. variegatus* eggs during early cleavage as a function of temperature and salinity. Means with the same letter are not significantly different ($P < 0.01$).

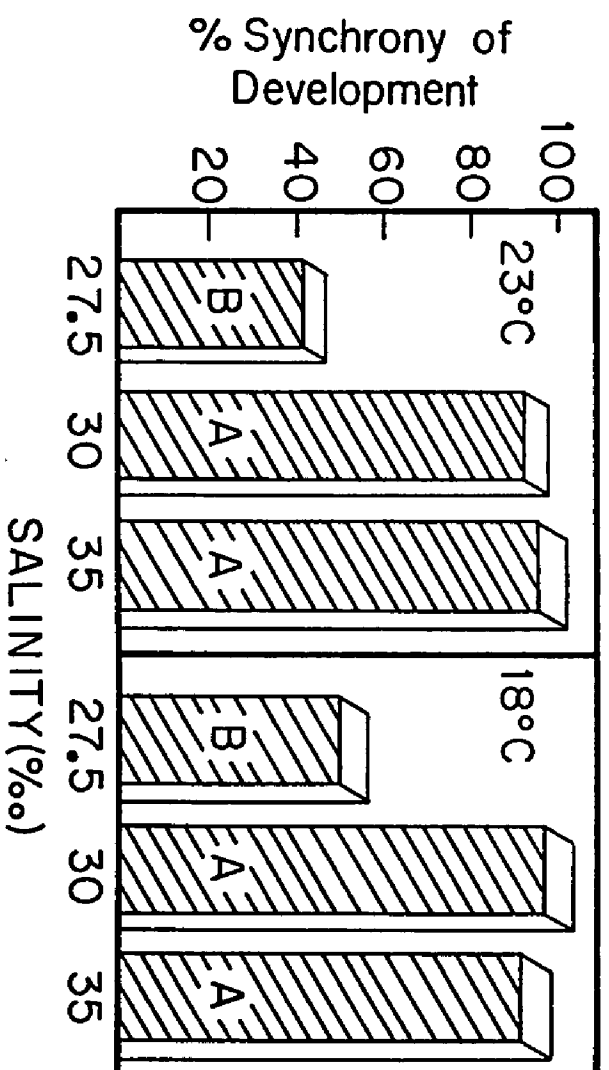


Figure 7. Light micrograph illustrating: (a) 8-arm L. variegatus plutei (w/primary podia) one hour prior to settlement and metamorphosis; (b) young juvenile urchin approximately 45 minutes after settlement and metamorphosis.

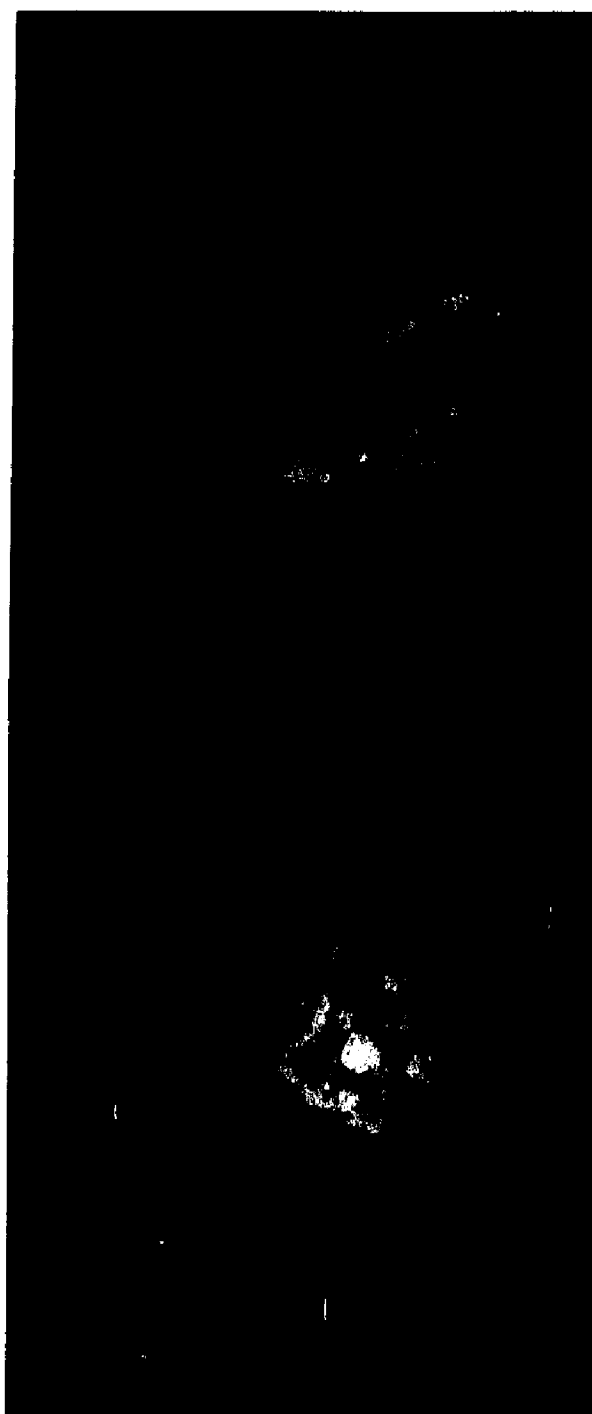


Figure 8. (a) Normal 8-arm pluteus (15 days old) cultured at 23°C and 35‰/ooS; (b) abnormal pluteus cultured at 23°C and 27.5‰/ooS and exhibiting reduced larval arms and visceral mass. The abnormal plutei did not feed.

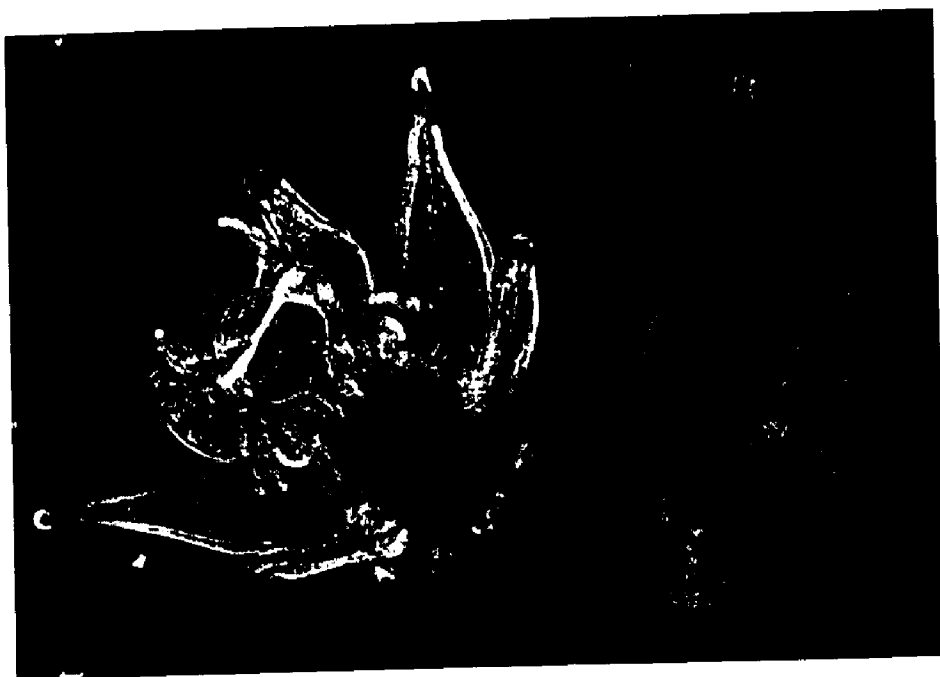


Figure 9. Light micrographs of 7 μ m sections of ovaries of S. droebachiensis and S. pallidus. (a) ovaries of S. droebachiensis acclimated to 30°/ooS for 4 weeks; (b) ovaries of S. droebachiensis acclimated to 20°/ooS for 4 weeks; (c) ovaries of S. pallidus acclimated to 30°/ooS for 4 weeks; (d) ovaries of S. pallidus acclimated to 20°/ooS for 4 weeks. Magnification= 120x.

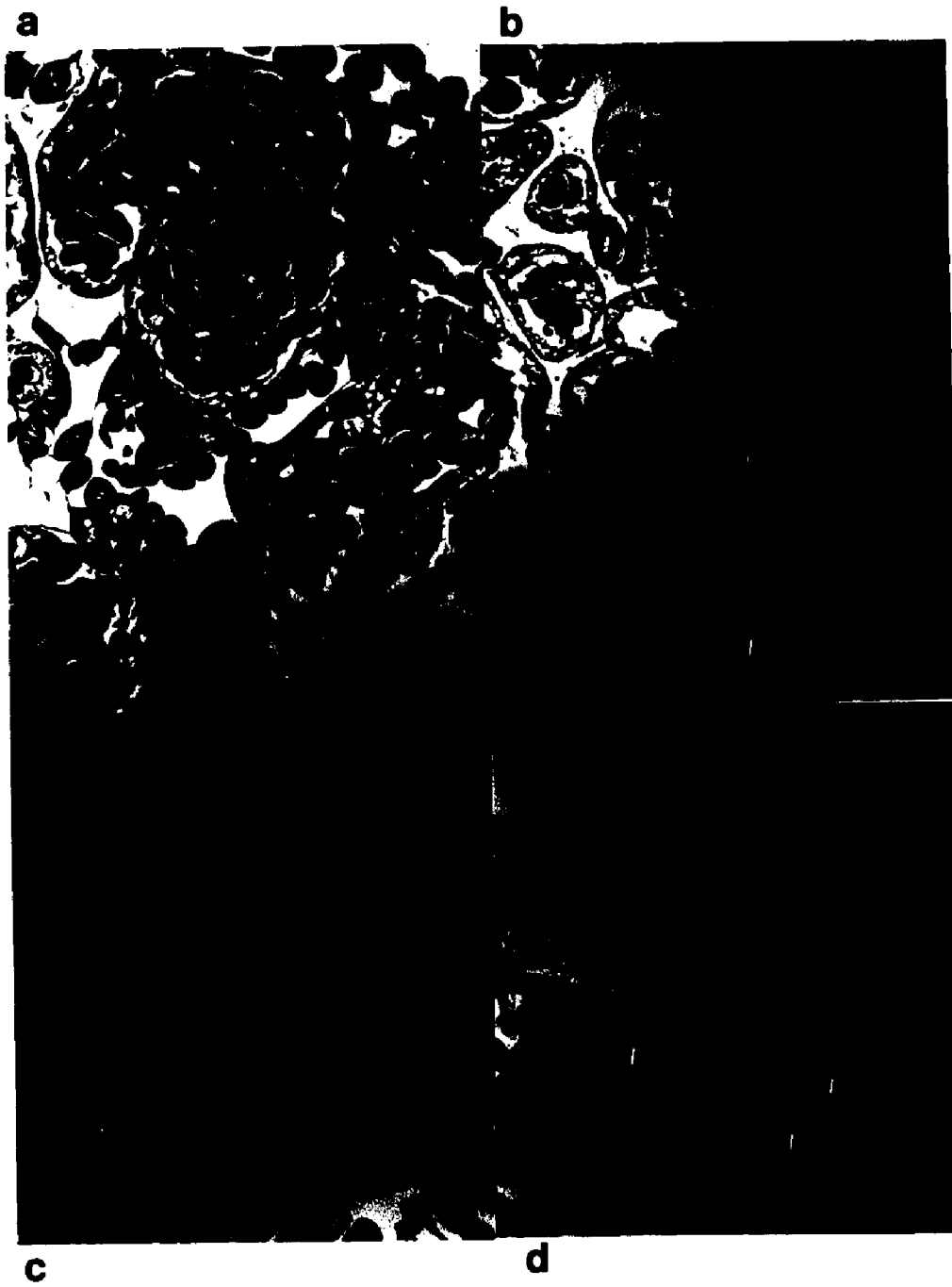


Figure 10. Total ninhydrin-positive substances (NPS) in ovaries of adult S. droebachiensis and S. pallidus acclimated to 30, 25, 20, and 17.5°/ooS for 1, 2, 3, and 4 weeks. Data are expressed as uM NPS/unit of relative cell volume in fertilized eggs.

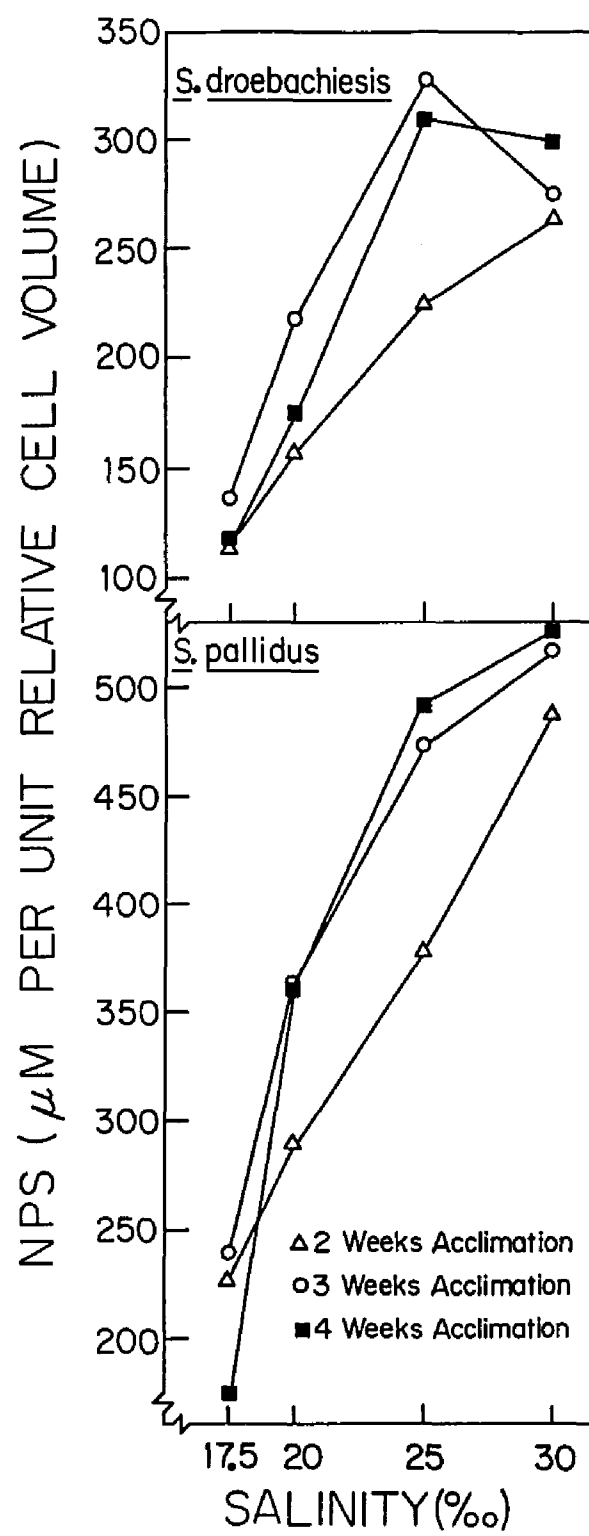


Figure 11. Percent fertilization of eggs of S. droebachiensis and S. pallidus after 6 hours exposure to various concentrations of lactic acid prior to fertilization (n=200). Means with the same letter are not significantly different ($P<0.01$).

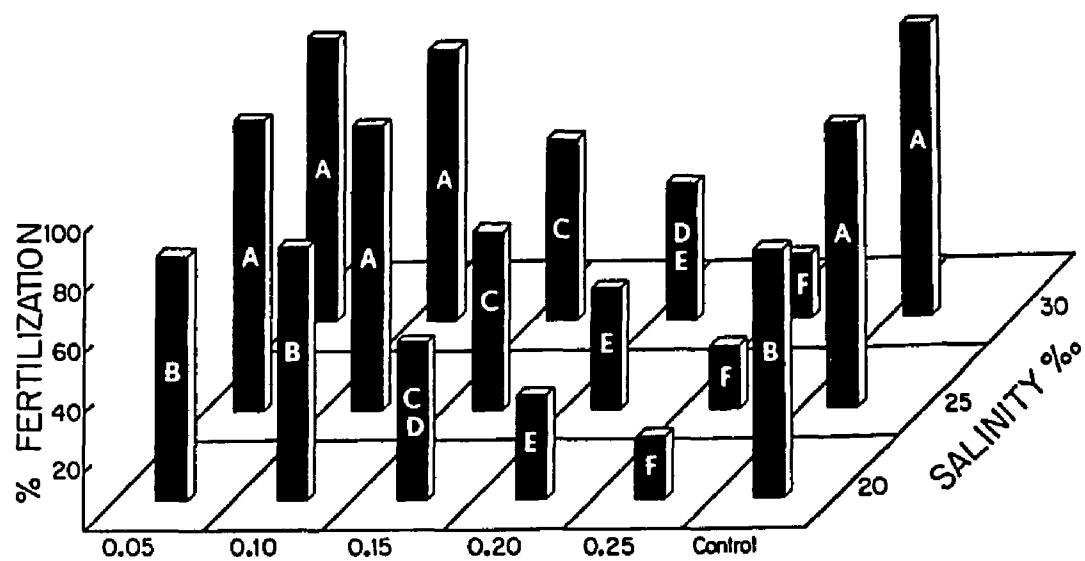
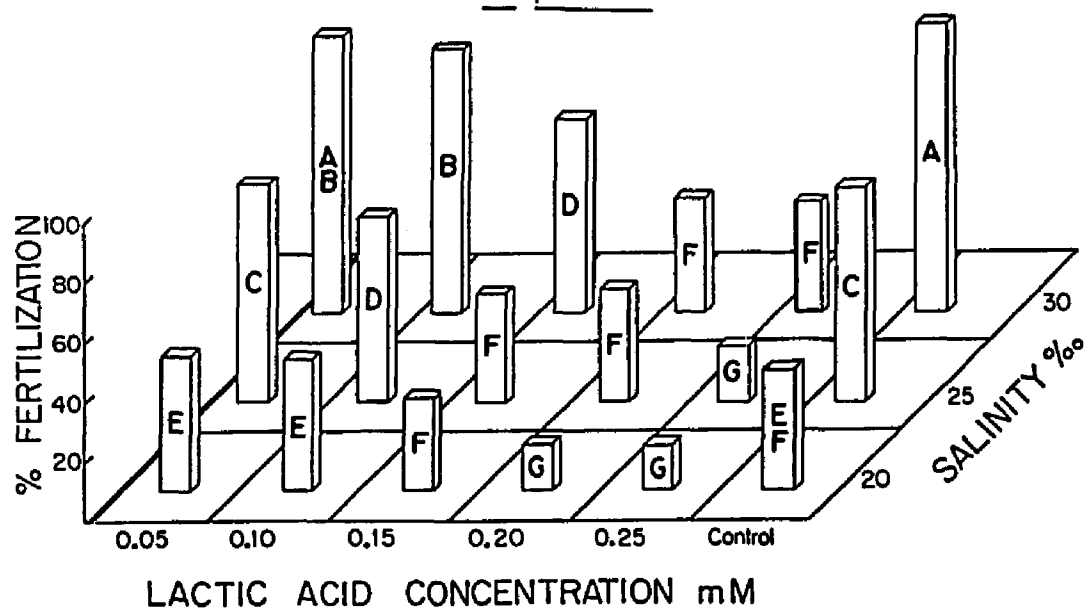
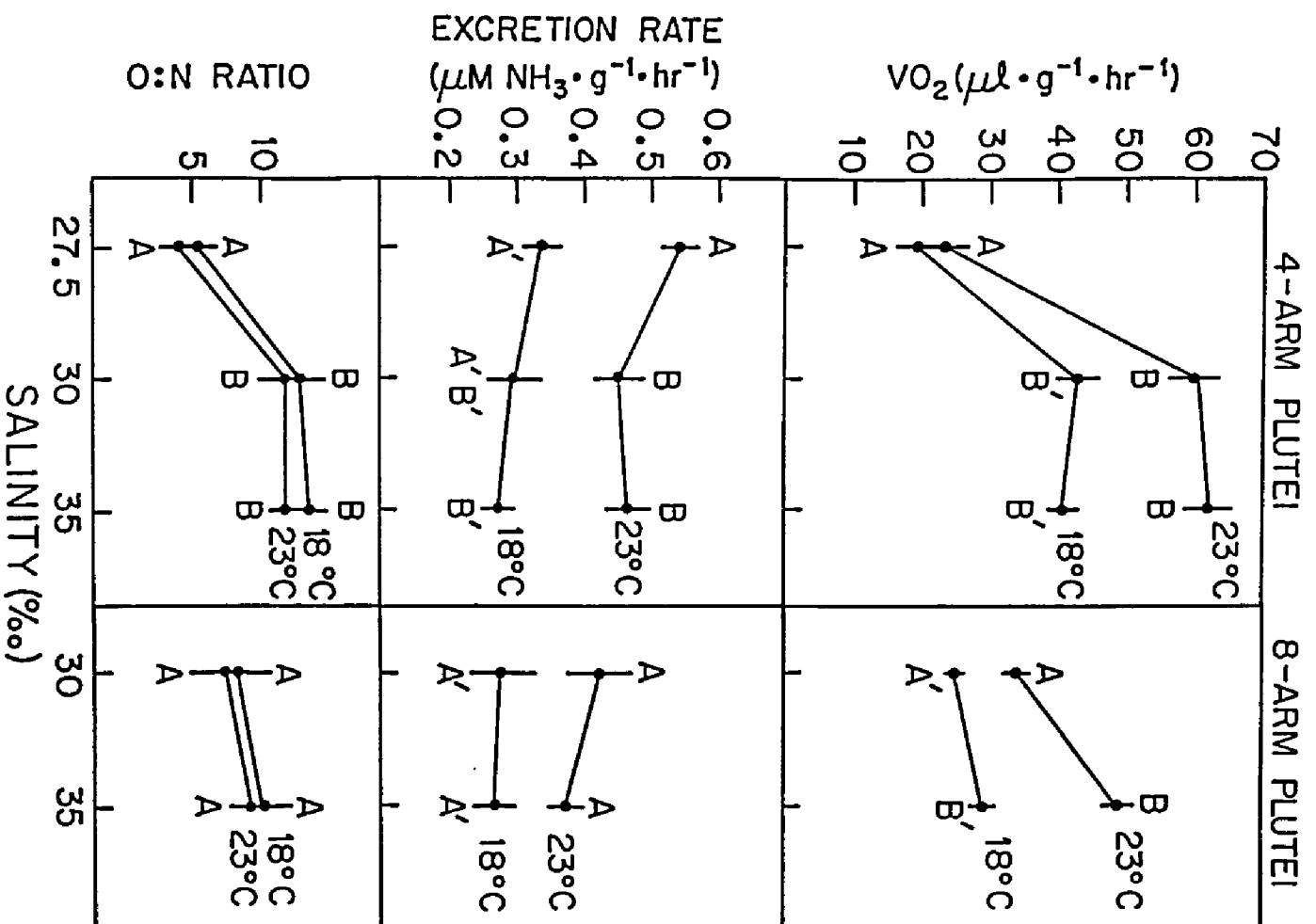
S. droebachiensis*S. pallidus*

Figure 12. Metabolic rates of 4 and 8-arm L. variegatus plutei as a function of temperature and salinity. Means with the same letter are not significantly different between salinities ($P < 0.01$). Letters indicated by a single quote (') are significantly different from letters without a quote between temperatures ($P < 0.01$).



SUMMARY

Populations of several echinoderm species are found in both brackish water (0.5-30⁰/ooS) and hypersaline (40-80⁰/ooS) environments (Kinne, 1964; Stickle and Diehl, 1987). The results of the present study illustrate that not only are adult echinoderms able to tolerate fluctuations in salinity; but that the larval stages are also capable of surviving some exposure to hyposaline environments. The salinity limits that marine invertebrate larvae are able to tolerate will in turn play a role in limiting the distribution of the species along salinity gradients; therefore, it is not surprising that the zone of lethality of the larvae of all species examined fall well within the observed tolerance limits of the adults along salinity gradients.

The duration of larval development will in turn determine the amount of time the larvae stay in the water column and therefore how long they are exposed to any variations in environmental factor gradients; in other words, the longer a larva remains in the plankton, the potential for high larval wastage (Thorson, 1950) increases. The larvae of P. ochraceus, S. droebachiensis, S. pallidus, and S. purpuratus are all found in cold waters (9.0-10⁰C), have a planktonic existence between 51-151 days, and are potentially dispersed over a wide geographic range (Strathmann, 1978). Larvae of these species could potentially have a greater risk of being exposed to fluctuating

environmental conditions than the larvae of a species with a much shorter planktonic existence such as L. variegatus.

The results of this research can be summarized as follows: (1) Salinity does indeed affect the developmental rates, metabolic rates, larval tolerances, and morphology of embryonic echinoderms. For the species observed, developmental rates and survival to metamorphosis decreased upon exposure to low salinity; (2) Respiration rates of L. variegatus varied directly with both temperature and salinity; whereas, excretion rates varied indirectly with salinity but varied directly with temperature. (3) Depressed O:N ratios at 27.5°/ooS for L. variegatus indicate physiological stress at low salinity; (4) Larval zones of lethality fell well within the observed tolerance limits of the adults; (5) Gonadal NPS values were significantly lower at 27.5°/ooS; whereas, coelomic cavity lactic acid levels were significantly higher at 20°/oo for S. droebachiensis and S. pallidus; (6) Histological examination revealed no observable differences in gonad structure between adult urchins acclimated to high and low salinity; (7) Therefore, pre-acclimation of adults to lowered salinity does not enhance larval tolerances or offer the embryos any apparent advantage in hyposmotic environments; (8) Observed larval salinity tolerances correlate well with known adult distributions along salinity gradients; (9) Newly settled juveniles possess no apparent advantage in low salinity waters when compared to adults or plutei. (10) Larval salinity tolerances may in fact limit the adult distribution of

echinoderms along salinity gradients. Few investigators have studied the effect of larval tolerance on the distribution of adult echinoderms along salinity gradients. The results of the present investigation suggest that larvae are more intolerant of low salinity than are adults. Most echinoderm biologists suggest that physiological constraints of adults prohibit entry into brackish water; therefore, further investigations should focus on the physiological capabilities of larvae with respect to salinity tolerances affecting population distribution.

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Department of Zoology/Physiology, 1987

Lecture Experience

Developmental Biology/Embryology, Mammalian Physiology, Comparative Physiology, Human Physiology, Environmental Physiology, General Biology, General Zoology, Invertebrate Zoology, Entomology

Membership in Professional Societies

American Society of Zoologists
 American Association for the Advancement of Science
 American Malacological Union
 Sigma Xi: The Scientific Research Society (Full Membership)
 Louisiana Society for Electron Microscopy
 Arkansas Academy of Science

Miscellaneous Experience

1. Eight years (1978-1986) active research experience in scanning electron microscopy (Cambridge S-600 and Hitachi S-500 SEMs) and transmission electron microscopy (RCA, Hitachi HU-11, and JEOL 100CX Tems).
2. President, Zoology & Physiology Graduate Student Organization (ZPGSO), Louisiana State University, 1984-1986

Honors and Awards

Deans List, University of Arkansas	1976-1980
National Deans List	1979-1980
Phi Kappa Phi Honor Society	1979
Martha Couch Givens Award in Biology, University of Arkansas	1980
National Register of Outstanding College Graduates	1980
Who's Who among Students in American Universities and Colleges	1980
R.A. LeFleur Fellow, Louisiana State University	1981-1986
Arceneaux Memorial Award for Research in Electron Microscopy	1982

Undergraduate Biology Courses

Biology, Microbiology, Botany, Vertebrate Zoology, Invertebrate Zoology, Mammalian Physiology, Microtechnique (Light, SEM & TEM), Histology, Embryology, Animal Behavior, Entomology, Plant Taxonomy, Natural History of Vertebrates, Genetics, Cell Biology, Comparative Vertebrate Morphology, Paleontology

Graduate Zoology/Physiology Courses

Protozoology, Comparative Physiology, Environmental Physiology of Estuarine Animals, Marine Ecology, Cell Physiology, Neurosensory Physiology, Histochemistry, Population Genetics, Scanning Electron Microscopy, Transmission Electron Microscopy, Advanced Cellular Techniques in Electron Microscopy, Invertebrate Embryology (Friday Harbor Laboratories), Larval Ecology (Friday Harbor Laboratories)

Miscellaneous Graduate Courses

Biochemistry, Experimental Statistics, SAS Seminar

Research Interest

Environmental Physiology and Embryology of Marine and Estuarine Animals

Financial Support

Sigma Xi Grant in Aid (National Headquarters- \$325.00; LSU Chapter- \$250.00) for dissertation research (1986)

Publications

Roller, R.A., D.W. Garton and W.B. Stickle. 1984. Regeneration of the proboscis, radula and odontophoral cartilage of the southern oyster drill *Thais haemastoma canaliculata* (Gray) (Prosobranchia: Muricidae) after amputation. American Malacological Bulletin 2:63-73.

- Garton, D.W., R.A. Roller and J. Caprio. 1984. Fine structure and vital staining of the osphradium of the southern oyster drill, Thais haemastoma canaliculata (Gray)(Prosobranchia: Muricidae). Biological Bulletin 167:310-321.
- Roller, R.A. and W.B. Stickle. 1985. Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms. Canadian Journal of Zoology 63(7):1531-1538.
- Roller, R.A. and W.B. Stickle. 1987. Intracapsular development of Thais haemastoma canaliculata (Gray)(Prosobranchia: Muricidae) under laboratory conditions. American Malacological Bulletin. (in revision).


DOCTORAL EXAMINATION AND DISSERTATION REPORT


Candidate: Richard A. Roller

Major Field: Zoology

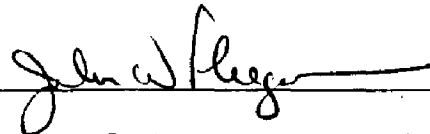
Title of Dissertation: Salinity Effects on the Development and Larval Tolerance of
Five Species of Echinoderms

Approved:

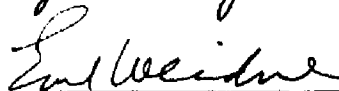

Major Professor and Chairman


Dean of the Graduate School

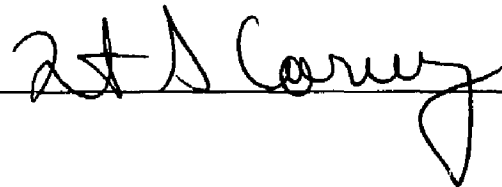
EXAMINING COMMITTEE:











Date of Examination:

July 7, 1987